HOSPITAL LIBRARY

VOL. 31 JULY, 1946 No. 7

PROPERTY OF THE
Kilmer Memorial Laboratory
of the Binghamton City Hospital
MUST NOT BE REMOVED
FROM LABORATORY

The Journal of Laboratory and Clinical Medicine

Clinical and Experimental

A Nutritional Survey of Starvation in a Group of Young Men. Charles S. Davidson, M.D., Boston, Mass., Major Harold L. Wilcke, Sn.C., and Private First Class Phillip J. Reiner, Army of the United States ---- 721

The Action of Thiobismol on Therapeutic Quartan Malaria. Captain Lawrence I Kaplan, Medical Corps, Army of the United States, Hilton S. Read, M.D., Atlantic City, N. J., and Frederic T. Becker, M.D., Duluth, Minn. ---- 735

The Distribution of Atabrine in the Blood, the Skin, and Its Appendages. Kurt Lange, M.D., and Milton J. Matzner, M.D., F.A.C.P., New York, N. Y. ---- 742

Pyribenzamine (N'-Pyridyl-N'-Benzyl-N-Dimethylethylenediamine HCl), an Antagonist of Histamine. R. L. Mayer, H. W. Hays, D. Brousseau, D. Mathieson, B. Rennick, and F. F. Yonkman, Summit, N. J. ---- 749

Studies on the Toxicity of Sulfonamide Drugs. I. Porphyrin-Excretion by a Patient Treated With Sulfadiazine and Later With Sulfanilamide. Frank H. J. Figge, Ph.D., T. Nelson Carey, M.D., and Glenn S. Weiland, Ph.D., Baltimore, Md. ---- 752

Observations on the Activity in Vitro of Succinylsulfathiazole and Phthalylsulfathiazole. H. J. Florestano, Ph.D., and J. F. Kennedy, Indianapolis, Ind. ---- 757

Fate of Sodium Ricinoleate After Oral Administration to White Rats. E. L. MacQuiddy, M.D., and Elizabeth Pinkerton, Ph.D., Omaha, Neb., and Hartmann Goetze, B.S., and Fred W. Oberst, Ph.D., Cincinnati, Ohio ---- 763

Observations on the Effect of Lowered Oxygen Tension on Sicklemia and Sickle Cell Anemia Among Military Flying Personnel. Major A. B. Henderson and Major H. E. Thornell, Medical Corps, Army of the United States, Detroit, Mich. ---- 769

The Bone Marrow in Hemophilia During Life. Louis R. Limarzi, M.D., Henry G. Poncher, M.D., and Carroll L. Birch, M.D., Chicago, Ill. ---- 777

An Unusual Case of Generalized Anasarca Appearing in an Impounded Dog With Cirrhosis of the Liver. Lillian Eichelberger, Ph.D., and Michael Roma, Chicago, Ill. ---- 785

(Contents continued on inside cover)

PUBLISHED BY THE C. V. MOSBY COMPANY, 3207 WASHINGTON BLVD., ST. LOUIS 3

Copyright 1946 by The C. V. Mosby Company

Sensitization of Rh-Positive Patients by the Rh Factor. Lester J. Unger, M.A., M.D., New York, N. Y. 793

The Traumatic Effects of Positive Intratracheal Pressures. Captain Howard Tatum and Lieutenant Colonel Arthur M. Ginzler, Medical Corps, Army of the United States 799

Laboratory Methods

An Apparatus for Measuring the Retained Dose of Inhaled Substances. Julian M. Tobias and Raymond E. Weston, Edgewood Arsenal, Md. 806

The Relative Efficiency of Water Centrifugal Sedimentation and Other Methods of Stool Examination for Diagnosis of Schistosomiasis Japonica. Second Lieutenant Bahij J. Baroody, Sanitary Corps, and Major Harry Most, Medical Corps, Army of the United States 815

A Presumptive Medium for Differentiating Paracolon From Salmonella Cultures. Martha L. Chilton, B.A., and MacDonald Fulton, Ph.D., Galveston, Texas .. 824

Book Reviews

Book Reviews 828

Editor

CARL V. MOORE, M.D.
Washington University School of Medicine
600 South Kingshighway
St. Louis 10, Mo.

Editorial Board

CLIFFORD J. BARBORKA, M.D.
Northwestern University, Chicago

PAUL R. CANNON, M.D.
University of Chicago, Chicago

RUSSELL L. HADEN, M.D.
Cleveland Clinic, Cleveland

GEORGE HERRMANN, M.D.
University of Texas, Galveston

JOHN A. KOLMER, M.D.
Temple University, Philadelphia

W. C. MacCARTY, M.D.
Mayo Clinic, Rochester, Minn.

T. B. MAGATH, M.D.
Mayo Clinic, Rochester, Minn.

EDWARD MUNTWYLER, Ph.D.
Long Island College of Medicine, Brooklyn

VICTOR C. MYERS, Ph.D.
Western Reserve University, Cleveland

M. H. SOULE, Sc.D.
University of Michigan, Ann Arbor

FREDERICK J. STARE, M.D.
Harvard University, Boston

ARNOLD D. WELCH, Ph.D., M.D.
Western Reserve University, Cleveland

Vol. 31, No. 7, July, 1946. The Journal of Laboratory and Clinical Medicine is published monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis (3), Mo. Subscription Price: United States, its Possessions, Pan-American Countries, $8.50 per year; Canada, $9.50; Foreign, $9.50. Entered as Second-Class Matter October 8, 1915, at the Post Office, at St. Louis, Missouri, under Act of March 3, 1879. Printed in the U. S. A.

A NUTRITIONAL SURVEY OF STARVATION IN A GROUP OF YOUNG MEN

CHARLES S. DAVIDSON, M.D.,* BOSTON, MASS., MAJOR HAROLD L. WILCKE, SN.C., AND PRIVATE FIRST CLASS PHILLIP J. REINER, ARMY OF THE UNITED STATES

AN OPPORTUNITY was afforded us during July and August, 1945, to observe starvation in a group of German prisoners in a section of Upper Austria. Because of the necessarily short time and the availability of only the simplest of field laboratory methods, the studies were limited. Nevertheless, during the period of starvation of these men, the amount of food eaten was known with considerable accuracy, so that interpretation of the clinical findings can be related to a fairly definite food intake.

The men were seen between two and three months after imprisonment, and during this interval the caloric value of the food eaten varied between 650 and 850 calories daily (see Dietary history under Results). Food eaten before the time of capture is not known and may not have been entirely adequate, so that before the beginning of the period of starvation the nutritional status of these men may not have been optimum.

MATERIALS AND METHODS

One hundred and seventy-one men, appearing to be the most severely undernourished of a larger group, were chosen for the examination. Most of the men had been occupied as heavy workers until they were unable to carry on because of lack of strength. Roughly 10 per cent of those studied were hospitalized because of malnutrition. There was no evidence of tuberculosis or of any acute infectious disease in any of the men included in this group.

The men ranged in age from 16 to 61; the average age was 31 (Table I). Fifty-five per cent were under 30 years of age, and only 6 per cent were over 50.

The method of conducting the survey was a modification of that developed for civilians by the Nutrition Division of the Office of the Surgeon General. The food issue was obtained, a brief physical examination was made on each individual, and blood hemoglobin and serum proteins were determined by the specific gravity method of Lowry and Hastings.[1] Apparatus and materials for these determinations were contained in the field laboratory kit developed by the Army's Medical Nutrition Laboratory. Age, height (in centimeters), and weight (in kilograms) were recorded and compared with standards for the United

Received for publication, March 6, 1946.

*Instructor in Medicine, Harvard Medical School; Assistant Physician, Thorndike Memorial Laboratory, Boston City Hospital; formerly, Consultant in Nutrition to the Surgeon General of the Army.

TABLE I. BODY WEIGHT REDUCTION BELOW STANDARD

	NUMBER	KG. BELOW STANDARD	% MORE THAN 10 KG. BELOW STANDARD
All	171	13	70
With edema	41	11	51
No edema	130	13	74
16 to 20 years	62	11	58
20 to 29 years	32	11	56
30 to 49 years	66	16	82
50 years +	11	15	82
Augsburg	994	6	18
Vienna	1,315	9	48

States.[2] The mean of the deviations from the American standards of all the individuals was determined. Two additional calculations also were made: first, the percentage of all the individuals who were more than 10 per cent below standard in weight; second, the percentage more than 10 kg. below standard in weight.

RESULTS

A. DIETARY HISTORY.—It was possible to calculate with a fair degree of accuracy the food intake of the 171 men during the two to three months of starvation, since the quantities of food issued were known, and it was impossible for the men to supplement the ration. We believe that the distribution was as even as it could be made, although at times an attempt was made to give the workers and those hospitalized for malnutrition more than their equal share of the food available. A sample menu is given in Table II. The calculation of the diet is only approximate, for the varieties of bread, meat, flour, cheese, and fresh vegetables changed from time to time. The caloric value of the food eaten varied between 650 and 850 calories daily during the two to three months considered here.

It may readily be seen that the diet was grossly deficient in calories, chiefly from the extremely low fat and carbohydrate content. Protein was low, but it may have been of high enough biologic value and sufficient in quantity to maintain nitrogen balance had the caloric value of the food been high enough. The calculated vitamin and mineral intake in the diet is certainly very low, and the former is probably higher than that in the food eaten, since most of the foodstuffs were cooked together in the form of a soup.

B. NUTRITIONAL STATUS.—

1. *General Appearance.*—Starvation was evident on inspection (Fig. 1). Most striking was the extreme loss of flesh, especially of body fat (Fig. 2). The usual deposits of body fat were gone, skin was loose, and it showed an almost complete absence of subcutaneous tissue. Posture was generally poor with stooped shoulders and kyphosis evident (Fig. 3). Weakness was obvious in many, and actual syncope occurred in several while waiting for physical examination. A characteristic lethargy was apparent in all of the men, being very severe in those appearing most malnourished. Facial expressions were dull and rarely changed.

TABLE II. APPROXIMATE COMPOSITION OF FOOD ISSUED DAILY

	GM.	CALORIES	PROTEIN (GM.)	CARBOHYDRATE (GM.)	FAT (GM.)	CALCIUM (MG.)	IRON (MG.)	SODIUM CHLORIDE (GM.)	VITAMIN A (I. U.)	VITAMIN B_1 (MG.)	VITAMIN B_2 (MG.)	NIACIN (MG.)	VITAMIN C (MG.)
Bread, black	100	240	10	49	1	24	2.3	1.7	0	0.2	0	1.3	0
Meat	50	70	10	1	3	7	1.0	0	26	0.04	0.1	4.3	0
Flour	30	104	3	23	0	9	1.5	0.04	7	0.1	0.05	0.9	0
Butter	10	81	0	0	9	2	0	0.2	425	0	0	0	0
Dried legumes	55	193	14	32	1	46	3.1	0.03	114	0.2	0.15	1.5	0
Fresh vegetables	50	8	1	1	0	39	2.0	0.06	5,000	0.08	0.14	0.4	10
Cheese	10	35	2	0	3	68	0	2	375	0	0.2	0.2	0
Sugar	15	60	0	15	0	0	0	0	0	0	0	0	0
Total		791	40	121	17	195	9.9	2.03	6,247	0.62	0.64	8.6	10

PROPERTY OF THE
Kilmer Memorial Laboratory
of the Binghamton City Hospital
MUST NOT BE REMOVED
FROM LABORATORY

2. *Complaints and Symptoms.*—

(a) Weakness and easy fatigability were spontaneous complaints in almost all of the men. They found that simple tasks such as arising from bed and walking a short distance required much effort, were difficult to accomplish, and produced undue fatigue requiring long periods for recovery. Most of the

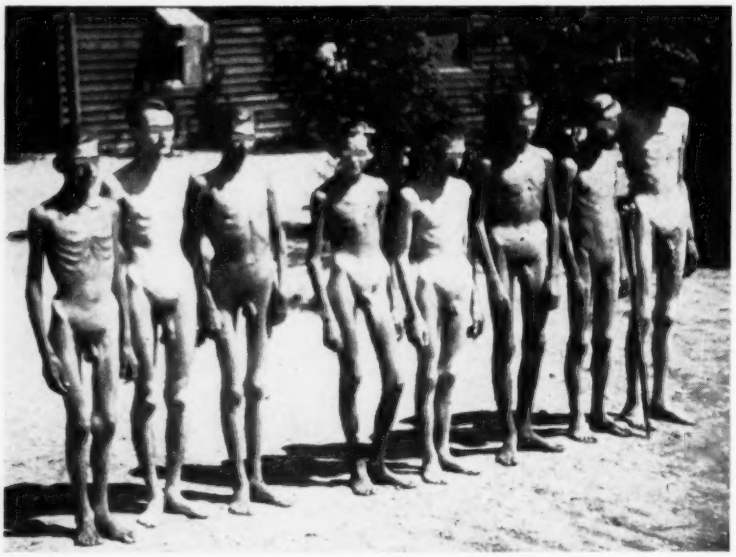

Fig. 1.

Fig. 2.

men remained in bed or were otherwise without activity most of the time. Vertigo and syncope were also common complaints, especially noticeable on arising from the horizontal position or when required to stand for even short periods of time.

(b) Pain in the hips, knees, and ankles, most prominently in the knees, was an extremely common complaint but bore no relation to the incidence of edema. The men observed this when walking, and often a prolonged period of rest was required for improvement. The pain appeared to be in the joints themselves. No joint abnormalities were observed on physical examination.

(c) Diarrhea was complained of by a number of men and was thought to be noninfectious in nature, but unfortunately no stool cultures could be done. Of the men with this complaint, many had several attacks, lasting for a day or so, each with free intervals of as much as two weeks.

(d) Paresthesias of the hands and feet were frequent complaints. This symptom will be discussed under Cardiovascular and Related Phenomena.

Fig. 3.

3. *Physical Examination.*—

(a) *Measurements:* Reduction in weight compared to United States standards[2] was present in all instances, the mean reduction being 13 kg. (Table I). Furthermore, 70 per cent of the men were more than 10 kg. below the standard. As might be expected, the younger age groups showed the least reduction, while of those over 30 years of age, 82 per cent were more than 10 kg. below the standard. Weight changes similarly obtained in the cities of Augsburg and Vienna are included for comparison. In Fig. 4 is shown the distribution of the percentage of body weight below the standard. It may be seen that 40 per cent of the group were more than 20 per cent below the standard; that is, for a 180-pound man a reduction of thirty-six pounds or more.

(b) *Integument:*

(1) Pigmentation. A variable amount of pigmentation of the skin was seen in many of the men. The pigmentation was most marked on the

exposed areas of the body, and most of the men had fair skin which tans easily. Nevertheless, certain characteristics of the pigmentation suggested that it was not entirely due to exposure. It was deeper in color and often appeared almost black. A few men seen elsewhere recovering from starvation showed a desquamation of pigmented skin, leaving a clear nonpigmented skin in its place. The pigmentation was not accentuated in the skin folds and creases, nor was it observed on the mucous membranes of the buccal cavity.

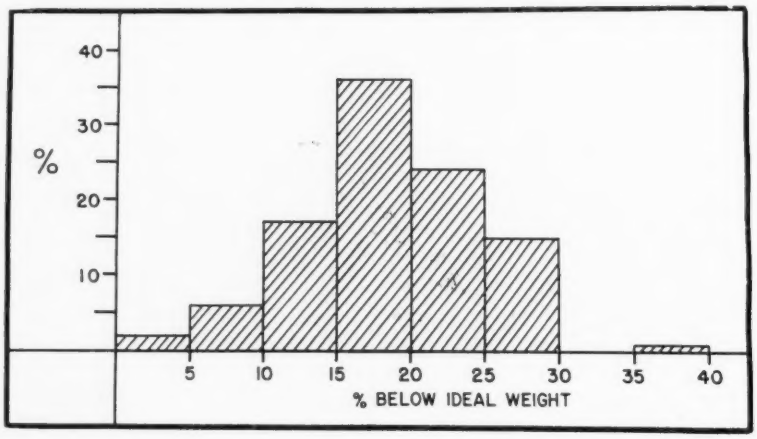

Fig. 4.—Distribution of weight reduction below standard expressed as percentage of standard or ideal.

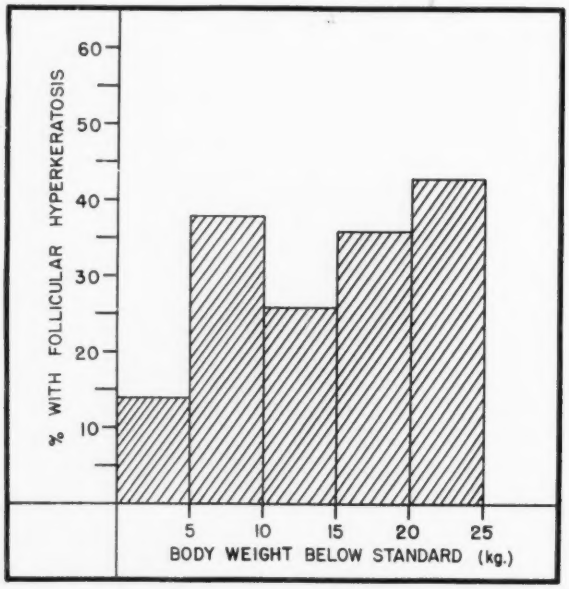

Fig. 5.—Incidence of follicular hyperkeratosis as related to reduction of body weight below standard.

(2) Follicular Hyperkeratosis. Fifty-four patients (32 per cent of the 171 men) were observed to have skin lesions similar to those described by Youmans and Corlette[3] and others and thought to be related to vitamin A deficiency. The lesions were usually present either on the extensor surfaces of the upper arms and frequently spread onto the shoulders or on the extensor surfaces of the thighs and buttocks, or, in extreme cases, both the upper and lower extremities were involved. There was found to be a rough parallelism between reduction in weight below the standard and the incidence of follicular hyperkeratosis (Fig. 5). Thus, of those patients who were 5 kg. or less below standard, only 14 per cent showed skin changes compared to 43 per cent for those from 21 to 25 kg. below standard.

(3) Sweat glands. A phenomenon occasionally seen in well-nourished individuals was observed in the men with pigmentation. Vesicles measuring from 1 to 5 mm. in diameter were scattered over the shoulders and upper trunk. They were easily broken and contained clear waterlike fluid. There was no reaction surrounding these lesions, which appeared to be sweat glands covered with a thin membrane of undesquamated skin. The men with these lesions said that in the sun other lesions appeared soon after one crop was broken.

(4) Other skin manifestations sometimes associated with deficiency disease. No instances of purpura, typical pellagrous dermatitis, or the so-called nasolabial seborrhea were seen. Scurvy was not seen.

(c) *Eye, Mouth, and Tongue Changes:*

(1) No instance of conjunctivitis was observed.

(2) There were four men with bilateral circumcorneal injection together with apparent ingrowth of capillaries into the cornea. These lesions resembled those described as due to riboflavin deficiency.[5]

(3) None of the corneal changes related to vitamin A deficiency were observed.[4]

(4) There was no instance of angular stomatitis and cheilosis as characterized by fissuring at the mouth corners and redness and denudation of the lips.

(5) There were only three men with change in the color of the tongue. In two, it was beefy red in color and in one, purple. None of these three showed papillary atrophy, but eight men did have moderate atrophy of the papillae of the tongue. None of these men was edentulous.

(6) Gingivitis was observed in a number of men, but in each instance it was associated with marked dental caries so that it could not be definitely associated with vitamin C deficiency. As already stated, there was no scurvy.

(d) *Cardiovascular and Related Phenomena:* Moderate hypotension and marked bradycardia were the rule (Fig. 6). The pulse rate and blood pressure were taken in sitting position but not at rest, as the determinations formed part of an active examination. Thirteen per cent of the men had pulse rates between 20 and 40 per minute; 71 per cent, between 41 and 60; 13 per cent, between 61 and 80; and only 3 per cent, above 80. There was no definite correlation between pulse rate and reduction in body weight below the standards.

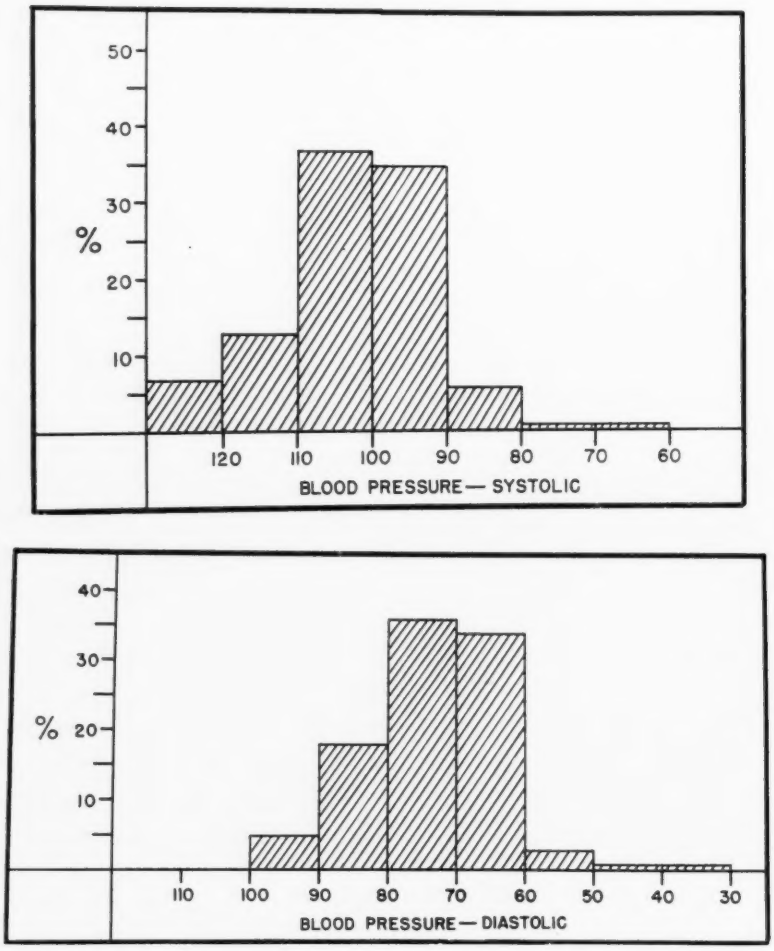

Fig. 6.—Blood pressure related to weight reduction below standards.

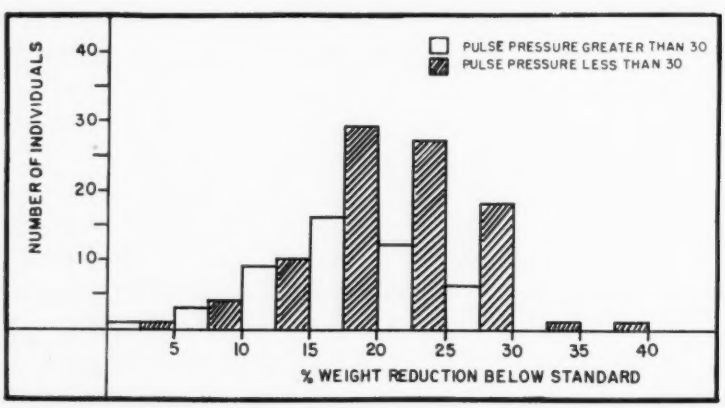

Fig. 7.—Relation between pulse pressure and percentage reduction in weight below standard.

J. LAB. & CLIN. MED.
VOL. 31, NO. 7, JULY, 1946

Fig. 8.

The blood pressure decrease was most marked in the systolic rather than the diastolic pressure, producing a diminished pulse pressure. In general, the pulse pressure was least in those men whose body weight was the greatest below the standard (Fig. 7).

Measurements of basal oxygen consumption were not possible. Realizing the inherent inaccuracies, calculation of the metabolic rate from Read's formula was done in thirty-two patients who, of course, were not under basal conditions. The metabolic rate so calculated ranged from –10 to –55 per cent, with an average of –28 per cent.

A phenomenon resembling acrocyanosis was observed in the skin of the hands up to the lower forearm (Fig. 8) and in the feet to just above the ankle. On a cool morning, these areas, particularly the hands, were cold to the touch and presented a mottled cyanotic appearance. During the warmer part of the day, the cyanosis sometimes gave way to a mottled reddish flushing, but the hands were still unusually cold to the touch. In a few instances there was a brownish pigmentation and occasionally a fine desquamation of the skin over the same areas.

This phenomenon resembling acrocyanosis was observed in 75 per cent of the men and generally was most marked in those patients with the greatest reduction in weight below the standard.

Sixty-five per cent of the men with the acrocyanotic appearance of the hands and feet complained of paresthesias consisting of numbness, tingling, or a burning sensation, most marked in the fingertips and toes but often extending into the hands and feet and even occasionally to the forearms and lower legs. Paresthesias of this variety were present in 24 per cent of the men examined. The acrocyanotic phenomenon suggested that the paresthesias were largely on a vascular basis rather than associated with peripheral neuritis or cord changes.

(e) *Extremities:*

(1) Nerve lesions. Calf muscle tenderness was observed in only three instances. There were two individuals with possible peripheral nerve palsy, one with peroneal palsy, unilateral, and the other with absent knee jerks. There were no other signs of thiamine deficiency in these two men. In all others the knee jerks and vibratory sense tested at the base of the great toes were present and apparently normal.

(2) Strength of grip. The ergometer was used to test the strength of the men in twenty-four instances. Each was given three trials; almost without exception, the first trial was the best, the second was much weaker, and on the third trial many of the men could barely move the needle. The best reading of the three was taken in each case and was essentially normal for the machine used. Significant only was the marked reduction on the second and third trials.

(3) Leg ulcerations. A number of the men had one or more leg ulcers, usually on the lower third of the leg. They were generally clean, but with little evidence of granulation, and had a punched-out appearance with sharp edges. The men said that these lesions were very slow in healing.

(f) *Edema and Serum Proteins:* There were forty-one men with varying degrees of dependent edema (Fig. 9). Many others who had been in bed

stated that they had swelling of the feet and ankles when they were up for an hour or so and that it as rapidly vanished when they reclined again. No sacral edema was seen.

The edema was of a soft, easily pitting character, always seen in the feet and ankles and sometimes as far up as the thighs. Only one man was seen with edema of the anterior abdominal wall. Several had evidence of ascites, as well as advanced leg edema. Edema of the face was occasionally seen and was most obvious in the eyelids, although many men volunteered that their face and eyes were swollen on arising in the morning.

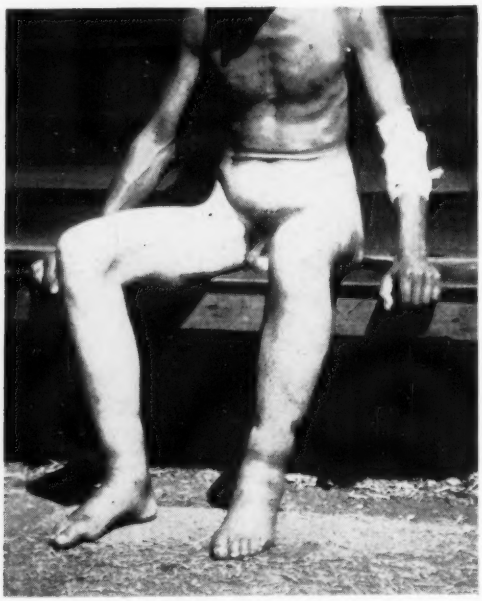

Fig. 9.

In general, the men with edema were of middle age, the young and the old being spared to a large extent. The mean reduction below the standard for body weight in persons with edema was 11 kg. (17 per cent) in contrast to 13 kg. for those without edema (Table I). Two factors appeared to account for the smaller reduction in weight. First, the body weight included a variable amount of edema fluid. Second, the men with edema often appeared to be better nourished than those without and were more likely to be of the stocky muscular type of build. In addition, there were many men with evidence of extreme weight loss but without any evidence or history of edema.

Fifty-one total serum protein determinations were made, eighteen of them on men with edema, with an average of 5.7 Gm. per 100 c.c. and with the lowest of 4.0 Gm. per 100 c.c. Thirty-six per cent of the serum proteins determined were 5.5 Gm. per 100 c.c. or below. Of the men without edema only 29 per cent were 5.5 Gm. per 100 c.c. or below, while of those with edema 47 per cent were in this low range (Fig. 10). Thus, whereas there were many men with

edema and normal proteins and men with low proteins and no edema, there was a definitely higher percentage of hypoproteinemia in those with edema. There was no correlation between weight reduction below the standard and serum protein concentration.

Signs which might be interpreted as indicating a deficiency of one or more of the members of the vitamin B complex were very few and were generally distributed evenly among those with and without edema. Most men with a

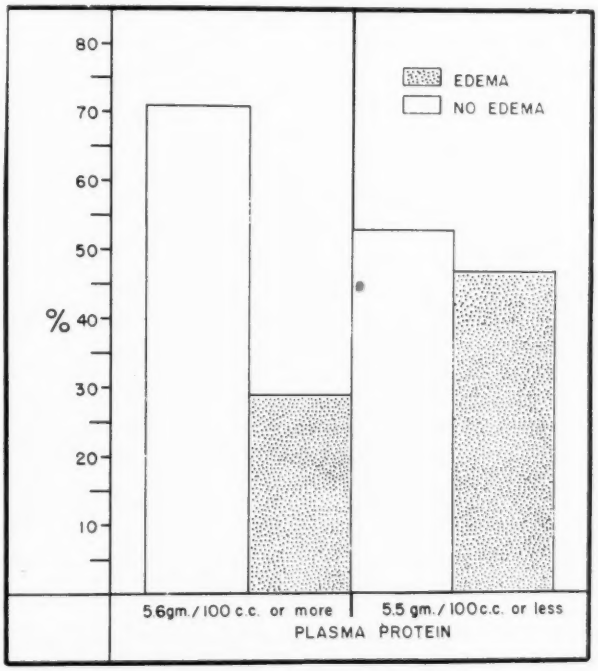

Fig. 10.—Incidence of edema in relation to serum protein level.

significant amount of edema complained of pain in the feet and ankles, and two of the three men with calf muscle tenderness had edema, suggesting a possible thiamine deficiency in these two men. Other than this possibility, there seemed to be no evidence pointing to vitamin deficiency as a cause for edema.

(g) *Anemia:* Sixty blood hemoglobin determinations and eighteen red blood cell counts were made. Only two men had hemoglobin concentrations below 10 Gm. per 100 c.c., or 64 per cent. Red cell counts were not done on these men. Eight of the eighteen red cell counts were below 4 million per cubic millimeter, but only one was below 3 million (2.9 million). This man had a hemoglobin concentration of 79 per cent and thus a color index of 1.4. This is the only instance of macrocytosis observed. The remainder of the color indices were from 0.8 to 1.1. Thus, it can be said that anemia was uncommon and when present was more likely to be normocytic and normochromic than hypochromic. Hemoglobin concentrations were no lower in those with edema than in those without. There was no definite correlation between reduction of body weight below standard and the hemoglobin concentration.

COMMENT

Starvation and malnutrition may be exhibited in quite a variety of ways in different individuals, depending on body stores of nutrients, environmental circumstances, and what foods are available for consumption. Starvation is primarily a deficiency of calories which results in a protein deficiency, because the body uses protein for caloric purposes. Vitamin deficiencies, particularly those of the water-soluble vitamins, may not be expected to be frequent in starvation because of the general decrease in metabolism. The men observed in this study had food intakes which showed evidence of deficiency almost exclusively of calories and protein.

Certain of the observations require special comment.

1. Pigmentation. It may be that the general skin pigmentation and related skin changes described were related only to exposure, but the distribution, intensity, and tendency to desquamation during recovery lead one to believe that other factors were concerned. There was no resemblance to the pigmentation of adrenal insufficiency or to pellagrous dermatitis. It is our opinion that the skin changes represent a slowing down of the usual skin replacement and desquamation, just as other body tissues are living at a slowed pace. Thus accumulation of skin rather than normal production and desquamation led to piling up of skin exposed to sun and the elements. The same explanation may account for the appearance in some of the men of vesicles representing sweat glands covered by undesquamated skin. The occurrence in these men of small, poorly healing leg ulcers may also represent a slowly reproducing skin rather than a specific vitamin deficiency. The low intake of vitamin C in these men, however, may have influenced the pigmentation,[6] the skin changes, and also the poor healing of the leg ulcers.[7]

2. Follicular hyperkeratosis. The incidence of this sign was certainly high in these men. The diet was very low in vitamin A, although it was fairly adequate considering carotene as provitamin A. The extremely low fat content of the diet undoubtedly impaired absorption of carotene and may have hindered conversion of carotene to vitamin A. Similar findings are mentioned in other reports on starvation. How much the lesions were related to a deficiency of this vitamin, and how much to lowered skin productivity as previously suggested, cannot be stated categorically. It is noteworthy that none of the eye signs described for vitamin A deficiency were observed.

3. Lesions resembling acrocyanosis. The lesions of the hands and feet resembling acrocyanosis and often associated with paresthesias were at once striking and difficult to explain. Similar changes of a milder degree are occasionally seen in thin adolescents. Individuals with peripheral neuritis as a rule have unstable neurovascular mechanisms in the affected parts, but there was very little confirmatory evidence of peripheral neuritis in these men.

Paresthesias and possible vascular changes in the hands have been ascribed to poor posture with drooping shoulders and pressure on the brachial plexus.[8] This may in part explain the phenomena observed in these men who certainly exhibited poor posture. Butler and co-workers[9] reported paresthesias in th

group of internees they studied, but they did not relate it to any specific deficiency. The high incidence of loss of vibratory sense found by these authors was not present in the group of men reported here nor in studies on starvation in Holland.[10]

4. The occurrence of "hunger edema" in 24 per cent of the men appears at first thought to be evidence of marked protein deficiency with hypoproteinemia. This was in part true; that is, there was a higher incidence of edema in those with hypoproteinemia than in those without (Fig. 8). Nevertheless, there were a significant number of men with edema but relatively normal serum protein concentrations. This lack of correlation between edema and the serum proteins in starvation has been observed by others.[10, 11] Several possible explanations appear for this. First, no partition between the albumin-globulin fractions was possible in this study, so that a hypoalbuminemia may have been masked by a hyperglobulinemia. Second, the edema in some of the men may have been associated with a thiamine deficiency. However, lack of other evidence of such a deficiency, the amount of thiamine in the diet, and the absence of signs of beriberi heart disease make this explanation unlikely. Reduction in blood volume in starvation also has been reported.[10] It has been suggested that this may mask a reduction in the total circulating serum proteins, but it is difficult to see how this could be related to edema formation since the plasma osmotic pressure is related to the concentration rather than to the total amount of serum protein. Furthermore, Keys, in experimental starvation in man,[11] reports only a small decrease in total blood volume made up of a proportionately greater reduction in volume of cells and an actual, although small, increase in plasma volume. At present the discrepancies between the occurrence of edema and the serum protein level need further clarification.

5. Anemia in these men was definite although not marked. In general it was normocytic and normochromic. As with the serum proteins, it may be thought that reduced blood volume masks a more severe anemia. However, the findings reported by Keys,[11] referred to previously, show a relatively greater reduction in red cell volume than in total blood volume.

6. The calculated (therefore, probably maximum) intake of these men of thiamine, riboflavin, niacin, and ascorbic acid, during the two or three months of starvation, is certainly low. The small incidence of signs classically related to deficiency of the water-soluble vitamins (B complex and C) suggests that the intake, although small, was sufficient during this time and with reduced caloric intake and general body metabolism to prevent specific deficiencies. For thiamine, Holt[12] suggests that between 0.24 and 0.44 mg. per 1,000 calories is protective. The requirements for riboflavin and niacin are less well known, but the intake in these men at this caloric level and for the time of starvation was sufficient to prevent gross signs suggestive of deficiencies of these vitamins. With respect to ascorbic acid, it is known that at least two months is required for the appearance of scurvy on a scorbutic diet. Thus, Crandon, Lund, and Dill,[13] in experimental human scurvy, found that over four months were required before the lesions appeared.

SUMMARY

1. A nutritional survey of 171 men suffering from a severe degree of starvation is reported.

2. The diet of these men averaged about 750 calories per man per day for from two to three months before the study.

3. Extreme wasting was evident upon inspection and was usually associated with lethargy, a stooped posture, weakness, and often actual syncope. Comparison of actual weight with data given in standard height and weight tables showed that all the men were under the standard in weight, the average being 13 kg. below the standard. Seventy per cent were more than 10 kg. below the standard.

4. Joint pains, prominently in the knees, were common.

5. Diarrhea of a recurrent variety was frequently a complaint.

6. A generalized pigmentation was observed.

7. Clinical evidence suggesting a deficiency of any of the vitamins was lacking except for the following: a high incidence of follicular hyperkeratosis, four instances of circumcorneal injection, three instances of color change in the tongue, and eight instances of papillary atrophy of the tongue. Eye signs suggesting vitamin A deficiency were not observed.

8. A condition resembling acrocyanosis was observed in the distal extremities of many of these men and was often associated with paresthesias.

9. Edema, probably of nutritional origin, was observed in 24 per cent of the men. Hypoproteinemia was common but was not present in a significant number of the men with edema.

10. Anemia was not severe, only two men having a hemoglobin concentration below 10 Gm. per 100 c.c. of blood.

REFERENCES

1. Lowry, O. H., and Hastings, A. B.: Histochemical Changes Associated With Aging. I. Methods and Calculations, J. Biol. Chem. **143**: 257, 1942.
2. War Department Training Manual 8-500, U. S. Government Printing Office, 1945.
3. Youmans, J. B., and Corlette, M. B.: Specific Dermatoses Due to Vitamin A Deficiency, Am. J. M. Sc. **195**: 644, 1938.
4. Blegvad, O.: Parenteral Vitamin Administration for Xerophthalmia, Ugesk. f. Laeger **85**: 942, 1923.
5. Sydenstricker, V. P., Sebrell, W. H., Cleckley, H. M., and Kruse, H. D.: The Ocular Manifestations of Ariboflavinosis; Progress Note, J. A. M. A. **114**: 2437, 1940.
6. Rothman, S.: In Vitro Studies on Pigmentation. II. Influence of Ascorbic Acid on Oxidation of Tyrosine by Ultraviolet Irradiation, J. Invest. Dermat. **5**: 67, 1942.
7. Bourne, G. H.: Vitamin C and Repair of Injured Tissues, Lancet **2**: 661, 1942.
8. Walshe, F. M. R.: On "Aeroparesthesia" and So-Called "Neuritis" of the Hands and Arms in Women, Brit. M. J. **2**: 596, 1945.
9. Butler, A. M., Ruffin, J. M., Sniffin, M. M., and Wickson, M. E.: The Nutritional Status of Civilians Rescued From Japanese Prison Camps, New England J. Med. **233**: 639, 1945.
10. Stare, F. J.: Nutritional Conditions in Holland, Nutrition Rev. **3**: 225, 1945.
11. Keys, A.: Personal communication.
12. Holt, L. E., Jr.: The Thiamine Requirement of Man, Federation Proc. **3**: 171, 1944.
13. Crandon, J. H., Lund, C. C., and Dill, D. B.: Experimental Human Scurvy, New England J. Med. **223**: 353, 1940.

THE ACTION OF THIOBISMOL* ON THERAPEUTIC QUARTAN MALARIA

Captain Lawrence I. Kaplan, Medical Corps, Army of the United States,
Hilton S. Read, M.D., Atlantic City, N. J., and
Frederic T. Becker, M.D., Duluth, Minn.

SCHWARTZ,[1] in 1939, first introduced sodium bismuth thioglycollate (thiobismol) as an effective agent for reducing the frequency of vivax malaria paroxysms without completely eliminating them. Other workers[2] subsequently confirmed the value of this drug in therapeutic malaria. Prior to this investigative work a variety of agents, including small amounts of quinine, arsenicals, and intravenous 20 per cent dextrose solution, was used unsuccessfully for regulating or temporarily interrupting malaria paroxysms.

Young, McLendon, and Smarr[3] clearly demonstrated that sodium bismuth thioglycollate in 0.1 or 0.2 Gm. amounts had an inhibitory effect against half-grown *Plasmodium vivax* parasites. Older or younger parasites were not affected in their series. Paroxysms, which were converted from quotidian to tertian periodicity, usually remained tertian for the duration of the infection and often through several subinoculations. The use of this drug, therefore, in the treatment of neurosyphilis with therapeutic malaria, allowed a full course of therapy without rapidly exhausting the patient from daily febrile elevations. It also facilitated temporary interruptions of malaria when these were indicated by minor complications.

Sodium bismuth thioglycollate was first used in quartan malaria by Young, McLendon, and Smarr,[3] in 1943, but no consistent results were obtained. Thirty-seven injections were administered to nine Negro patients showing either one, two, or three broods of *Plasmodium malariae* parasites. Occasional, unpredictable interruptions occurred, but no correlation of the patients' previous cycles, the time of administration of the drug, the clinical effect, or the effect on the parasitemia was offered.

Attention was directed first to the action of thiobismol on quartan malaria at this Neurosyphilis Center, when an attempt to interrupt temporarily the vivax parasitemia in a patient experiencing a mixed *P. vivax* and *P. malariae* infection resulted in the drug's unexpected elimination of the quartan parasitemia with little effect on the vivax cycle. In retrospect, it was evident that, at the time of injection, the vivax parasites were mature and, therefore, according to previous studies, not susceptible. The action of sodium bismuth thioglycollate on quartan malaria, however, was further investigated. Two and three daily injections, respectively, were given to two patients undergoing quartan malaria therapy. Both experienced prolonged interruptions, with afebrile periods lasting for more than sixteen days. Since this prolonged

From the Neurosyphilis Center, Finney General Hospital, Thomasville, Ga.
Received for publication, March 7, 1946.
*Thiobismol, sodium bismuth thioglycollate.

effect was not particularly desirable, only single injections of the drug were used in the remainder of the study.

The purpose of this investigation was twofold: (1) to determine whether sodium bismuth thioglycollate inhibits half-grown *P. malariae* trophozoites, an action similar to that observed in vivax malaria, and (2) to study the regulating effect of the drug in irregular quartan malaria cycles, noting especially the optimal time of administration for the predictable interruption of individual paroxysms.

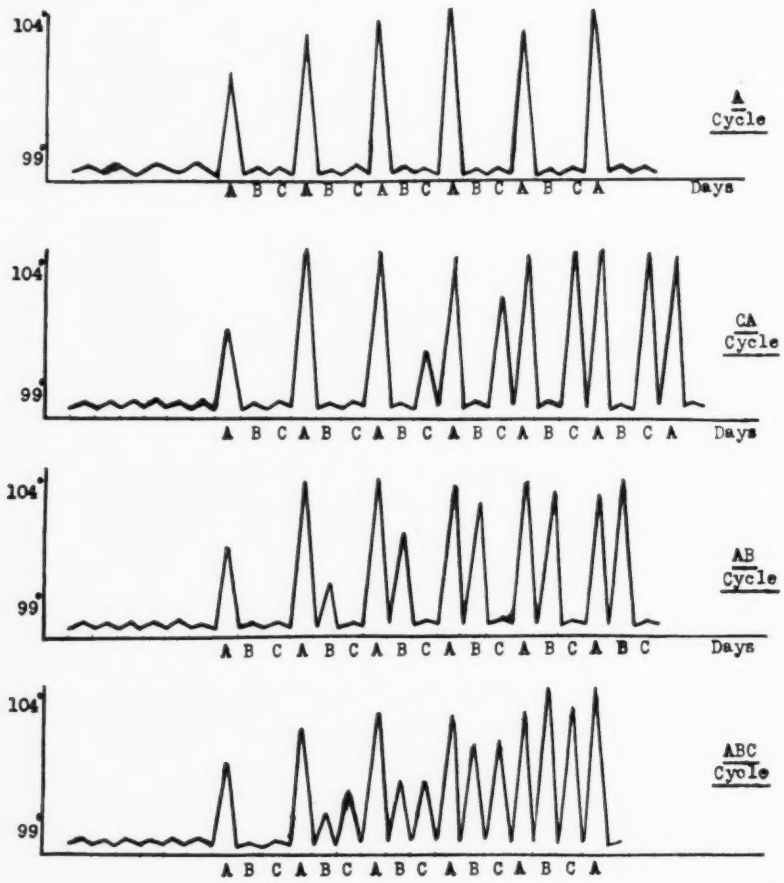
104°
99°
A Cycle
A B C A B C A B C A B C A B C A Days
CA Cycle
A B C A B C A B C A B C A B C A B C A Days
AB Cycle
A B C A B C A B C A B C A B C A B C Days
ABC Cycle
A B C A B C A B C A B C A B C A

Fig. 1.—Examples of temperature graphs observed with the various quartan malaria cycles.

MATERIAL AND METHOD

Fifty-six injections of sodium bismuth thioglycollate were administered to thirty-eight patients (six white, thirty-two Negro) in this series. Three of the thiobismol injections were given in doses of 0.1 Gm. and the remaining fifty-three in doses of 0.2 Gm. Quartan malaria was induced routinely by blood inoculation intravenously with varying parasite dosages. The quartan cycles encountered varied from the true quartan cycle with one paroxysm occurring every third day to the quotidian quartan cycle with a paroxysm occurring daily. The thiobismol used* was injected intramuscularly in all instances. No

*A preparation of Parke, Davis & Company, Detroit, Mich.

arbitrary schedule of injections was followed, for it was thought that chance distribution over the period of seventy-two hours from the onset of the main quartan paroxysm would reveal more pertinent information.

Quartan malaria usually produces a single paroxysm every third day, two daily paroxysms alternating with one fever-free day, or three daily paroxysms with no fever-free day. Occasionally, more irregular courses with poor delineation of paroxysms may occur. The main cycles have arbitrarily been designated by most investigators as true quartan (one paroxysm every three days), double quartan (two paroxysms every three days), and quotidian quartan (three paroxysms every three days). Those paroxysms appearing in a true quartan cycle are usually referred to as the *A* paroxysms or cycle. Parasites causing the *A* cycle, that is, maturing during the *A* paroxysms, were designated the *A* brood. Subsequent intermediate paroxysms on the second or third day following the main cycle have been termed the *B* and *C* paroxysms, respectively. These different quartan cycles with their *A*, *B*, and *C* designations are illustrated graphically in Fig. 1. Quartan malaria, therefore, ordinarily presents one of four types of cycles: *A* (true quartan), *AB* (double quartan), *CA* (double quartan), and *ABC* (quotidian quartan). The *BC* type of cycle, in which the main *A* paroxysms disappear later in the febrile period, is rarely seen and was not encountered in this series. These cycle descriptions have been employed frequently in this paper, since they simplify the subject matter and often indicate the effects of thiobismol more precisely.

TABLE I. CLINICAL EFFECTS OF SODIUM BISMUTH THIOGLYCOLLATE ON QUARTAN MALARIA RELATED TO TIME OF ADMINISTRATION, AGE OF PARASITE BROODS, AND PREVIOUS CYCLES

TIME* OF ADMINISTRATION	AGE OF BROODS†			TRUE QUARTAN A				DOUBLE QUARTAN AB			DOUBLE QUARTAN CA			QUOTIDIAN QUARTAN ABC					TOTAL
	A	B	C	A REMOVED	INTERRUPTION‡ A UNAFFECTED	NO EFFECT	TOTAL	A REMOVED	B REMOVED	TOTAL	A REMOVED	PROLONGED INTERRUPTION§	TOTAL	A REMOVED	B REMOVED	B AND C REMOVED	NO EFFECT	TOTAL	
±6	Mature	⅔	⅓	–	5	6	11	–	4	4	–	–	–	–	–	1	–	1	16
7 to 18	⅙	⅚	½	–	1	–	1	–	–	–	–	–	–	–	–	–	–	–	1
19 to 30	⅓	Mature	⅔	2	6	8	16	1	–	1	–	3	3	–	–	–	2	2	22
31 to 42	½	⅙	⅚	2	–	2	4	–	–	–	–	–	–	1	–	–	–	1	5
43 to 54	⅔	⅓	Mature	1	–	5	6	–	–	–	2	–	2	1	–	–	–	1	9
55 to 66	⅚	½	⅙	–	–	2	2	–	–	–	–	–	–	–	1	–	–	1	3
Total				5	12	23	40	1	4	5	2	3	5	2	1	1	2	6	56

*Calculated as the number of hours from the onset of the main *A* paroxysm.
†Listed as fractions of maturity, that is, ½ equals half-grown parasites.
‡Interruption for from four to eight days with reappearance of the *A* cycle.
§Prolonged interruption for from sixteen to thirty days after injection.

In contrast to patients with vivax malaria, in whom, at any particular time, there are two different age groups of parasites undergoing schizogony, patients with quartan malaria present three different stages of parasites at one time. Therefore, to determine the regulating effect of sodium bismuth thioglycollate in quartan malaria, the growth stage of the parasite broods causing each of the cycles must be correlated with the time of administration of the drug. This has been done in Table I. For example, twenty-four hours after the onset of the main *A* paroxysm, the *A* parasites are one-third grown, the *B* parasites mature, and the *C* parasites two-thirds grown. The time of administration of thiobismol in this study was calculated as the number of hours from the onset of the main *A* paroxysm.

The following data was tabulated on each patient:

1. Dose of sodium bismuth thioglycollate.

2. Previous cycles (*A*, *AB*, *CA*, or *ABC*) and number of paroxysms experienced prior to injection.

3. Time of administration of sodium bismuth thioglycollate.

4. Effect on parasitemia (drop in parasite count per cubic millimeter listed).

5. Clinical effect, indicating the number of afebrile days following the injection, the subsequent cycle of paroxysms, and the designation of eliminated cycles.

6. The occurrence of an out-of-cycle paroxysm immediately following the injection.

RESULTS AND DISCUSSION

Forty of the fifty-six injections of sodium bismuth thioglycollate were administered to patients experiencing *A* cycles only (Table I). Definite clinical interruptions occurred in seventeen (42.5 per cent) of these forty patients. Two types of clinical effect were noted. In five patients (12.5 per cent) sodium bismuth thioglycollate caused a temporary interruption lasting from four to eight days, followed by the appearance of a *B* or *C* cycle of paroxysms with the original *A* cycle eliminated. In the remaining twelve patients (30 per cent), of those experiencing definite clinical effects, the temporary interruption of from four to eight days was followed by the reappearance of the *A* cycle with no intermediate paroxysms.

Of eleven injections given to patients at the time when the *A* brood of parasites was mature, five (45.5 per cent) resulted in temporary interruption, but the *A* cycle was unaffected and reappeared. Of four injections given when *A* parasites were half-grown, two (50 per cent) were followed by temporary interruptions with the subsequent appearance of a *B* or *C* cycle and the elimination of the original *A* cycle. In the other patients with definite clinical effects, *A* cycles were also removed when the *A* brood of parasites was one-third and two-thirds grown, and temporary interruption, without affecting the *A* cycles, occurred when they were one-sixth and one-third grown. Although it is obvious that, in patients with true quartan malaria, a definite clinical effect following the injection of sodium bismuth thioglycollate can be expected only irregularly, the parasites inhibited were uniformly partially grown. Little or no effect was observed on cycles produced by mature or very young parasites.

Sixteen injections of sodium bismuth thioglycollate were administered to patients experiencing irregular quartan cycles of the *AB*, *CA*, and *ABC* types. Of these, five were given to patients with the *AB* double quartan cycle. A definite clinical effect followed each of these five injections. In three patients a temporary interruption of from three to six days occurred with a true quartan cycle (*A* or *B* only) supervening. In the remaining two patients there was no sustained interruption of paroxysms, but a single paroxysm was removed, converting the cycle to a true quartan one (*A* or *B*). Four of the five injections were administered when the *A* parasites were mature and the *B* two-thirds grown. The *B* cycle was removed in each of these instances, and the *A* cycle was unaffected. One of the five injections was given when the *B* cycle was mature and the *A* one-third grown, and this resulted in removal of the *A* cycle only. Although injections were given only at these times in the patients with *AB* double quartan malaria, partially grown trophozoites were always inhibited, and no effect was observed on mature parasites.

Five injections were given to patients experiencing the *CA* type of double quartan malaria, and all of these were followed by definite clinical effects. In

three patients prolonged interruption of paroxysms for more than sixteen days occurred. Because of the limitation on the patients' hospitalization and the fact that they had already completed the prescribed course of therapeutic malaria, they were placed on routine atabrine therapy before the return of paroxysms could be noted. In the other two patients temporary interruption of paroxysms for from three to four days ensued, and the subsequent cycle was converted to the true quartan type. These latter two patients received thiobismol when the *A* parasites were two-thirds grown and the *C* parasites mature, and in both instances the *A* paroxysms only were removed. In the three patients experiencing prolonged interruption, the injections were given when the *A* parasites were one-third grown and the *C* parasites two-thirds grown. Thus, the prolonged interruption may have been occasioned by the partial growth of both broods of parasites with simultaneous inhibition by thiobismol. It is probable that paroxysms would have returned had the period of observation been prolonged further.

Of the six injections given to patients experiencing quotidian quartan types of cycle (*ABC*), four were followed by appreciable clinical effects. One, administered when the *A* parasites were mature, the *B* two-thirds grown, and the *C* one-third grown, resulted in the elimination of the *B* and *C* cycles, with conversion to true quartan malaria with the *A* cycle only. Two of the six injections given when the *A* parasites were half-grown and two-thirds grown, respectively, resulted in the elimination of the *A* cycle and conversion of the quotidian course to double quartan malaria of the *BC* type. One other injection, administered when the *B* parasites were half-grown, resulted in the elimination of this cycle and the conversion to a *CA* type of double quartan cycle.

The remaining two of the six injections, given to patients when the *A* parasites were one-third grown, the *B* parasites mature, and the *C* two-thirds grown, resulted in no clinical effect. Although, on the basis of the previous results, inhibition of the partially grown *A* and *C* parasites was expected, the failure may have resulted from the height of the parasitemia in these patients. Immediately prior to the injections of thiobismol, the parasitemia in one patient was recorded as 17,120 per cubic millimeter and in the other as 33,630. These parasite counts were the highest in the entire series of thirty-eight patients. The usual parasite density in quartan malaria encountered in this series ranged from 2,000 to 6,000 parasites per cubic millimeter. In spite of the fact that clinical effects were not observed in these two failures, the parasitemia was markedly affected, with a drop in parasite density within thirty-six hours of the injection to 6,336 per cubic millimeter in one case (40 per cent of the original parasitemia) and to 5,670 in the other case (15 per cent of the original parasitemia). This further evidence would permit one to suppose justifiably that a clinical effect would have resulted had the parasitemia originally been at the usually lower level. Thiobismol, therefore, apparently has a predictable, uniform action in reducing the frequency of paroxysms in double quartan or quotidian quartan malaria cycles, if the parasitemia, prior to injection, is below 10,000 parasites per cubic millimeter.

An analysis of the effect of sodium bismuth thioglycollate on the *P. malariae* parasitemia further supports this conclusion. In Table II is listed the percentage drop in parasite density, correlated with the original height of the parasitemia and the presence or absence of clinical effects following injection. It is noted that none of the patients experiencing definite clinical effects had original parasite counts above 10,000 per cubic millimeter, while five (20 per cent) of the twenty-five injections not followed by clinical effects were given to patients showing high parasitemias ranging from 11,120 to 33,630 per cubic millimeter. These high parasite densities, therefore, although they may be appreciably reduced by the injection of thiobismol, may not reach low enough densities to occasion clinical interruption. It is also interesting to note, however, that two cases showing definite clinical effects were not accompanied by appreciable decreases in parasitemia.

TABLE II. EFFECT OF SODIUM BISMUTH THIOGLYCOLLATE ON P. MALARIAE PARASITE DENSITIES RELATED TO THE CLINICAL EFFECTS AND ORIGINAL PARASITEMIAS

		DEFINITE CLINICAL EFFECT				NO CLINICAL EFFECT				
	ORIGINAL DENSITY†	0 TO 4	5 TO 10	OVER 10	TOTAL	0 TO 4	5 TO 10	OVER 10	TOTAL	TOTAL
Drop in *Parasitemia**	0 to 20	7	4	-	11	-	-	1	1	12
	21 to 40	8	4	-	12	1	2	1	4	16
	41 to 60	1	2	-	3	1	4	2	7	10
	61 to 80	2	1	-	3	1	1	1	3	6
	81 to 100	1	1	-	2	8	2	-	10	12
	Total	19	12	0	31	11	9	5	25	56

*Drop in parasitemia calculated as percentage of original parasite density, that is, drop to 5,000 per cubic millimeter from original density of 10,000 per cubic millimeter equals 50 per cent drop in parasitemia.

†Original parasite density in thousands of parasites per cubic millimeter.

In 75 per cent of all instances when sodium bismuth thioglycollate was injected on fever-free days, a paroxysm, usually reaching from 102 to 104° F., was precipitated within from one to four hours following the injection. Such an effect was, of course, masked when the drug was administered during the febrile episode. The previous demonstration that sodium bismuth thioglycollate in quartan malaria as well as in vivax malaria inhibits partially grown trophozoites suggests that the inhibited parasites may prematurely break out of erythrocytes and cause out-of-cycle temperature elevations. At any rate, no correlation between the precipitation of paroxysms and the occurrence of clinical interruption or reduction in frequency of paroxysms was observed.

SUMMARY AND CONCLUSIONS

1. Fifty-six injections of sodium bismuth thioglycollate in doses of 0.1 and 0.2 Gm. were administered at different intervals to thirty-eight patients experiencing true, double, and quotidian types of therapeutic quartan malaria.

2. Only 42.5 per cent of the injections given to patients with true quartan malaria were followed by temporary interruption. However, in all of these cases showing definite clinical effects, only partially (one-third, one-half, or two-thirds) grown parasites were inhibited.

3. Eighty-seven and five-tenths per cent of the injections administered to patients with double quartan or quotidian quartan malaria resulted in temporary interruption and/or reduction in frequency of paroxysms. Partially grown parasites were uniformly affected in these patients, and no mature or very young parasites (less than one-third grown) were influenced by the injections.

4. None of five patients with original parasite densities above 10,000 parasites per cubic millimeter responded to sodium bismuth thioglycollate injections with definite clinical effects. Two of these patients (12.5 per cent of the series of double quartan and quotidian quartan malaria patients) had the highest parasite densities in the entire series. In these instances the injections of thiobismol were followed by an appreciable decline in parasite count, but no reduction in frequency of paroxysms occurred.

5. Sodium bismuth thioglycollate appears to be an effective drug for reducing the frequency of paroxysms and regulating the febrile cycles in patients experiencing double quartan or quotidian quartan malaria. Its use may enable patients with neurosyphilis to tolerate better complete courses of therapeutic quartan malaria.

REFERENCES

1. Schwartz, W. F.: The Effect of Thio-Bismol on Therapeutic Malaria, J. Pharmacol. & Exper. Therap. **65**: 175-184, 1939.
2. Brunsting, L. A., and Love, W. R.: The Tempering Effect of Sodium Bismuth Thioglycollate (Thio-Bismol) on Therapeutic Malaria, Proc. Staff Meet., Mayo Clin. **15**: 285-288, 1940.
3. Young, M. D., McLendon, S. B., and Smarr, R. G.: The Selective Action of Thio-Bismol on Induced Malaria, J.A.M.A. **122**: 492-494, 1943.

THE DISTRIBUTION OF ATABRINE IN THE BLOOD, THE SKIN, AND ITS APPENDAGES

Methods for the Rapid and Simple Detection of the Presence of Atabrine in Blood, Skin, and Nails

Kurt Lange, M.D., and Milton J. Matzner, M.D., F.A.C.P.
New York, N. Y.

THE importance of an effective and safe antimalarial therapy can hardly be overestimated. Since the introduction of atabrine (Quinacrine hydrochloride, U. S. P. XII) many attempts have been made to make this drug more effective by establishing quickly an effective plasma level for a sufficiently long period of time, for we know that its plasmodicidal effect in vitro is directly dependent upon its concentration.

This undertaking, however, is dependent upon a reliable method to determine atabrine levels in the blood readily. This would enable one to evaluate clinically the feasibility of at least a temporary cure of this disease by establishing a safe and effective atabrine blood level.

The methods used so far have been rather complicated. The older methods[1-3] are not sensitive enough to permit control of the plasma level during suppressive treatment and even not during therapeutic attempts. While the studies described in this paper were in progress, Brodie and Udenfriend[4] published a method which is both sensitive and highly accurate. It is, however, a rather complicated and detailed procedure requiring the skill of a chemist and a well-equipped laboratory. At the same time Masen[5] published a method which is less complicated, although less sensitive, but still is rather intricate and requires considerable laboratory facilities. After our extraction method was completed, but unpublished due to the general ban on publications concerning malarial therapeutics, a report by Auerbach and Eckert[6] appeared which described a somewhat similar but improved method.

Since it is well known that atabrine may cause an intense yellow staining of the skin if taken over long periods of time in suppressive doses or in some individuals after one course of therapeutic application, it was interesting to see whether the level of atabrine in the blood was in any way related to the level in the skin which can be determined also by photofluorometric means. The Dermofluorometer developed by one of us (K. L.)[7] for the purpose of determining the fluorescein content in the skin after an intravenous injection of this dye can be used also with slight changes to determine the content of atabrine in the skin as long as it is fluorescent. This skin fluorescence also may be observed visually by the use of a long-wave ultraviolet light source, thus enabling the observer to judge the distribution of atabrine in the skin and its appendices.

From the Department of Medicine, New York Medical College, Flower and Fifth Avenue Hospitals, and the Metropolitan Hospital (Research Unit).

Aided by a grant from the John and Mary R. Markle Foundation.

A paper with essentially the same content was submitted for publication in the early part of 1944 but was not published on account of the ban on publications on malarial therapeutics then in existence.

Received for publication, April 15, 1946.

It was the aim of this investigation to find a method for plasma level as well as skin level determinations which could be employed at outlying stations by an average laboratory assistant without the need of extensive laboratory facilities. At the same time it should be accurate enough that by the use of a more sensitive measuring instrument it would be of sufficient accuracy for laboratories with more elaborate facilities.

PROCEDURE

From 15 to 20 c.c. of venous blood are drawn and transferred into a glass-stoppered bottle which contains 2 drops of heparin solution. Plasma and red cells are separated by centrifugation in a graduated test tube to determine the hematocrit. Seven cubic centimeters of plasma are then pipetted into a glass-stoppered, no-lub separatory funnel of 60 c.c. capacity. Two cubic centimeters of 4 N sodium hydroxide solution and 7 c.c. of absolute ether (C.P.) are added. If the fluorophotometer is used, 14 c.c. of ether are added resulting in a ratio of 1:2. The separatory funnel is stoppered and shaken vigorously for four minutes. After standing ten minutes, the aqueous layer is let off and discarded. The supernatant liquid is transferred into an appropriate cuvette which is stoppered to avoid evaporation.

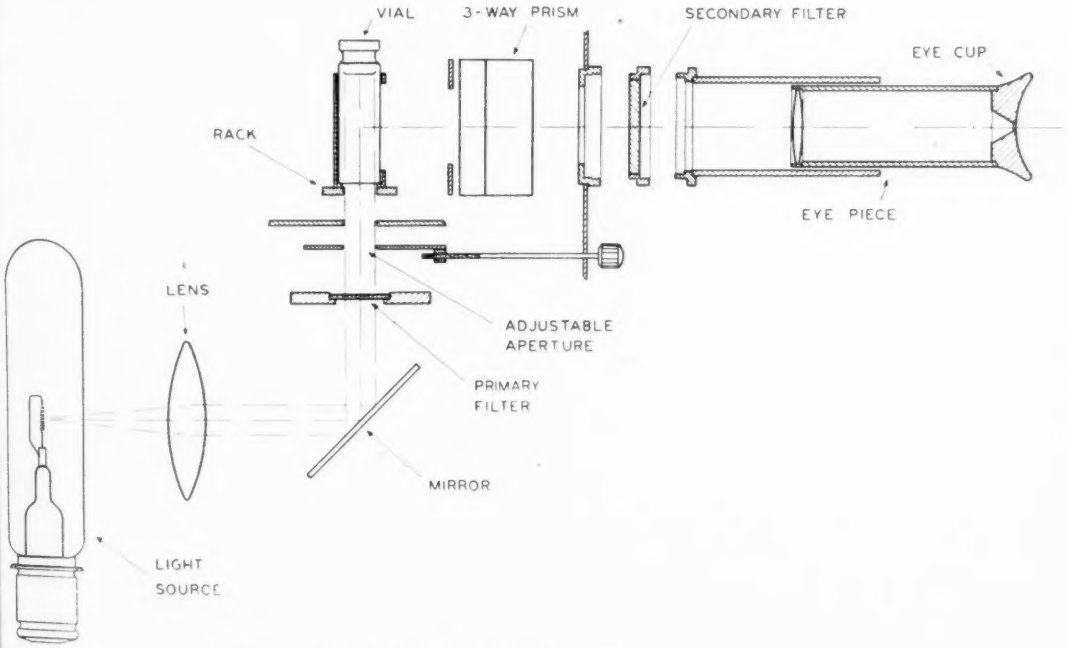

Fig. 1.—Schematic setup of the optical fluorescence comparator.

One of two types of fluorescence meters may be used in this determination: a fluorophotometer of the direct reading type* with special equipment for small samples or a simple visual fluorocomparator recently devised by us.[8] The fluorocomparator consists of a special incandescent bulb which casts its light from a close distance through a lens and a filter of Corning glass No. 5543 on to a mirror arrangement which reflects the beam on to three matched cuvettes (Fig. 1). The fluorescence thus excited is read through a selective filter of Corning glasses No. 352 plus No. 4303.

*Lumetron No. 402 EF, Photovolt Corporation, New York, N. Y.

The same filter combination is used in the fluorophotometer. This "narrow band" filtering of the secondary filter excludes undesired fluorescence of substances other than atabrine which would otherwise have to be removed by complicated extraction procedures.

The unknown is rapidly read in the instrument against an ether extract containing 0.5 gamma atabrine per cubic centimeter which is set to 100 on the slide wire. This extract is prepared by making a 1:10 dilution of a stock atabrine solution (50 mg. atabrine in 2,000 c.c. of phosphate buffer of pH 7.4). Five cubic centimeters of the diluted stock solution are shaken for four minutes with 25 c.c. absolute ether (C.P.) and separated as described. A blank consisting of an ether extract of the previously mentioned buffer solution alone is set to zero. Since there has been a 1:2 dilution in the preparation of the ether extract, ten divisions on the slide wire now correspond to 0.1 gamma per cubic centimeter.

The accuracy and specificity of the method are shown in Table I, the data for which were obtained by carrying on the following control procedures.

TABLE I

AMOUNT OF ATABRINE ADDED (GAMMA PER CUBIC CENTIMETER)	ATABRINE RECOVERY FROM		ATABRINE CONTENTS OF RESIDUE
	BUFFER SOLUTION (PER CENT)	PLASMA (PER CENT)	
0.5	100	98	0
0.4	97	100	0
0.3	98	97	0
0.2	100	96	0
0.1	98	101	0
0.075	97	97	0
0.05	98	97	0
0.025	102	96	0
0.015	97	102	0
0	0	0	0
0		Plasma icteric 0	
0		Plasma lipemic 0.015 gamma Unspecific fluorescence	
0		Plasma icteric 0	
0		Plasma high protein content	

The watery residue was read after the extraction and was found to give the same result as plain buffer, indicating that no atabrine was left in the residue.

Corresponding amounts of atabrine were added in each instance to 10 c.c. of plasma and 10 c.c. of buffer extracted with the ether. The readings were found to be the same as the readings of the buffer-atabrine solution within the range of ±3 per cent, thus proving the complete recovery from plasma.

Plasma specimens from ten patients with different diseases, especially with high icterus indices and with lipemia, but no atabrine treatment, were extracted and the readings were found to be equal, or very close to equal, to extracts from plain buffer, indicating that the fluorescence obtained with this method is specific for atabrine and atabrine derivatives.

The plasma levels of twenty-two patients suffering from relapses of vivax malaria were studied for their atabrine content during therapy. It was evident after a few observations that the therapeutic plan starting the patients on from 0.3 to 0.6 Gm. of atabrine the first day, followed by 0.2 Gm. daily for seven days, entails an unnecessary delay in the desirable rise in plasma levels (Fig. 2). Although the optimal atabrine plasma level has not been universally accepted, it appears that the maintenance of a plasma level of at least 100 μg per 1,000 c.c. is

desirable for a therapeutic effect. The initial dose should be large enough to raise the plasma concentration to that height within the first twenty-four hours. Most of the patients obtaining an initial dose of 1.2 Gm., followed by 0.6 Gm. the second day, to be followed by 0.3 Gm. throughout the course, showed this result (Fig. 3). This same experience was described by Shannon and his coworkers.[9]

The skin of patients under atabrine treatment is known to assume frequently a yellowish tint. Since this is related to atabrine deposits in the skin, efforts were made to study the degree of fluorescence by exciting it with ultraviolet radiation of a specific wave length. One may observe to what extent optically active atabrine is deposited in the skin and whether or not this is proportional to the atabrine plasma levels.

PROPERTY OF THE
Kilmer Memorial Laboratory
of the Binghamton City Hospital
MUST NOT BE REMOVED
FROM LABORATORY

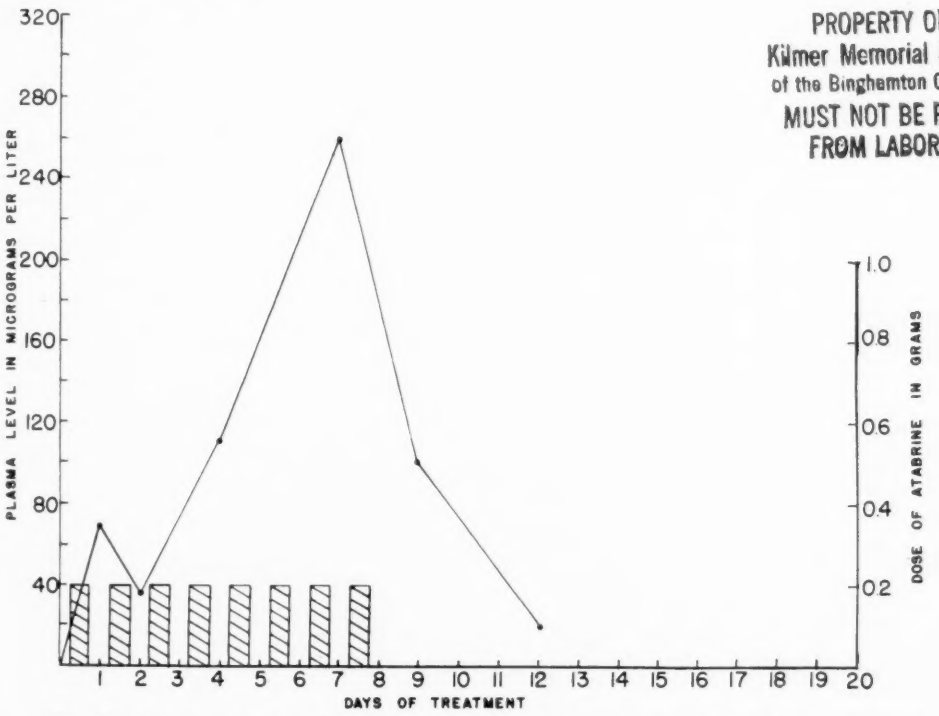

Fig. 2.—L. J. treated according to an old scheme with equal doses of atabrine for seven days. The plasma level rises very slowly, and a satisfactory therapeutic level is obtained only for a short time.

A mercury vapor bulb* is covered with a double filter of Corning glass No. 587. This lamp was used by one of us (K. L.)[10] for fluorescein studies and found to be very effective to demonstrate traces of fluorescence.† If this long-wave ultraviolet light of the main wave length of 3600 Angstrom units is beamed on the skin of the patients during or after atabrine treatment, one will discover that many of them, especially under extended treatment, show a specific greenish-white, jadelike fluorescence of the nails, the palms of the hands, and the plantae

*EH 4 General Electric.
†Supplied by G. W. Gates and Co., Franklin Square, L. I.

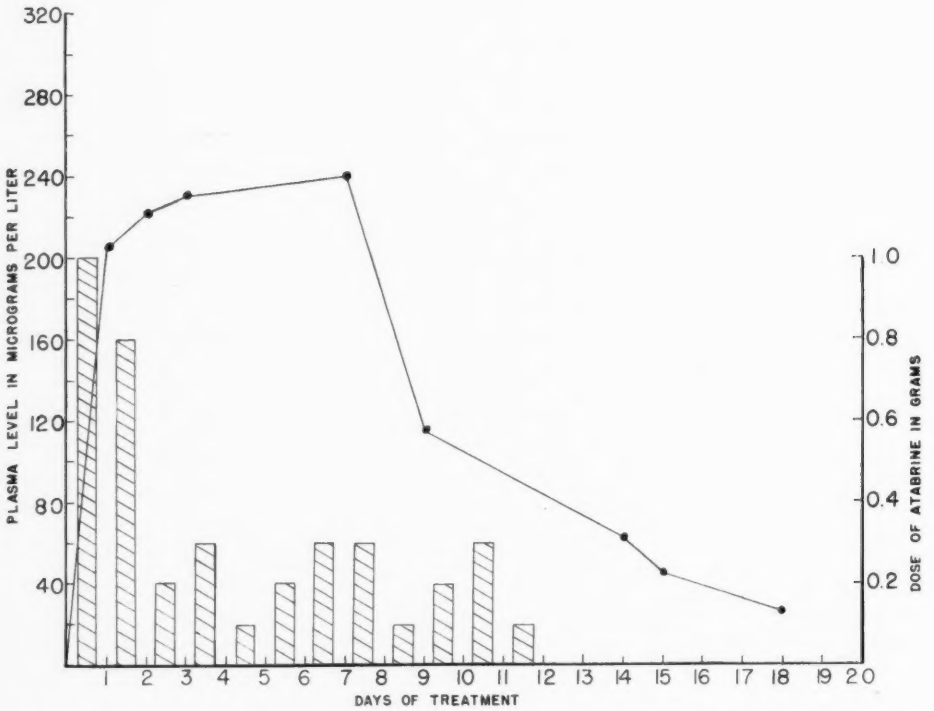

Fig. 3.—L. H. treated with high initial doses. The plasma level rises rapidly to a therapeutic range and stays there for several days.

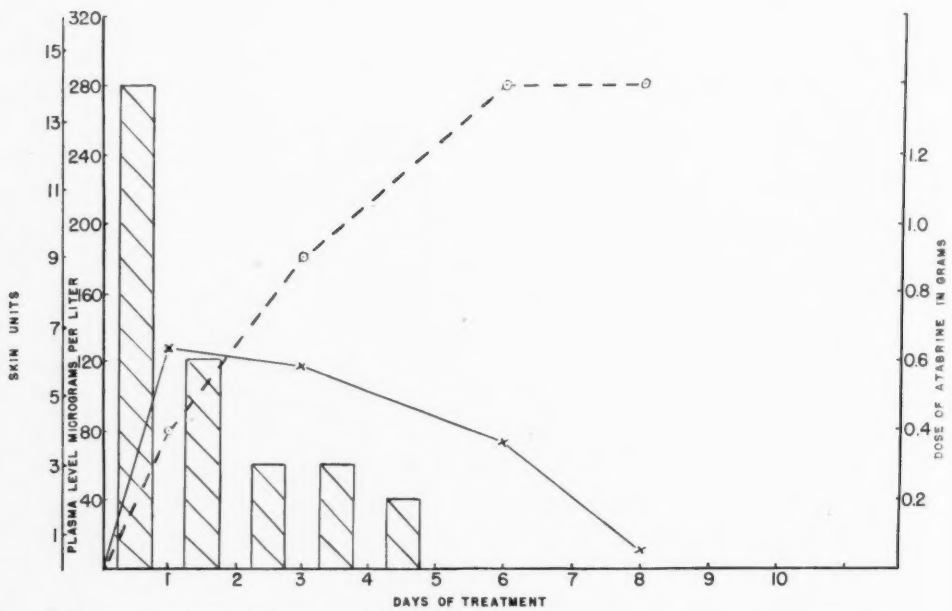

Fig. 4.—Relation between blood and skin levels of atabrine during atabrine therapy.

of the feet, while the skin itself appears in a verdigris color. Once this typical color is familiar to the observer, he can immediately identify the presence and the degree of fluorescence due to atabrine in the skin. The degree of the fluorescence can be measured quantitatively with the Dermofluorometer which we described earlier.[7] This photoelectric instrument specifically registers the fluorescence in the skin, and the amount of atabrine in the skin can thus be measured quantitatively.

In all twenty-two patients in whom the atabrine plasma level and the skin level were determined daily or at least every other day, no direct correlation between those two factors could be established. While the atabrine content in the plasma rose to its highest levels on the second or third day of therapy, the skin level in most patients rose slowly day by day as long as the therapy was continued. (Fig. 4.) It even continued to rise in the majority of patients for from two to five days after discontinuation of the drug, while the plasma level returned completely or almost completely to zero. It is, therefore, evident that the skin level of atabrine, as determined by the Dermofluorometer, does not correspond to the absolute concentration of atabrine in the blood. We have, however, never seen a case in which the skin level did not rise considerably under therapy. It is not necessary to use the Dermofluorometer to establish the presence of atabrine in the skin, for it can easily be detected visually by the use of the long-wave ultraviolet lamp. The presence of skin fluorescence as an isolated observation does not permit one to differentiate whether it has resulted from present or past administration.

During therapy atabrine is deposited through the matrix into the nail substance. The nail thus assumes an intense jadelike color under ultraviolet light if it contains atabrine. By observing the distribution of fluorescence in the nail, one is able to infer the periods during which atabrine therapy was employed. The amounts given with suppressive treatment are fully sufficient to produce the fluorescence of the nail. With cessation of therapy the blood level drops to zero and deposition of the drug in the nail ceases, thus permitting the nail which is just deposited under the matrix to be free of the specific atabrine color. Thus in an individual who has gone through several courses of therapy or suppressive treatment, one is able to see on the nails, under ultraviolet light, bands of jadelike color in comparison with the purple appearance of the normal nail. Knowing the approximate speed of growth of the nail, one can estimate how long a person has taken the drug and how long he has discontinued taking it.

The specific fluorescence of the nails under long-wave ultraviolet light permits one to state whether or not a person is taking the drug, since in all persons taking atabrine, a deposit will appear in the nails. This would be of importance in regular checks to establish whether the personnel is willfully failing to take the ordered medication.

SUMMARY

1. A rapid simple method to determine atabrine levels in the plasma for the effective treatment and possible suppression of malaria is desirable.

UNIVERSITY OF MICHIGAN LIBRARIES

2. A simple extraction method for atabrine from plasma using ether, which can be carried out without elaborate laboratory facilities, is described.

3. A previously described fluorocomparator was found satisfactory for clinical estimations of the plasma level of atabrine.

4. Skin levels of atabrine can be estimated visually by means of a special small ultraviolet lamp or measured quantitatively by a photoelectric Dermofluorometer.

5. Clinical studies in twenty-three patients under atabrine therapy reveal that:

(a) There is no direct correlation of skin and plasma levels of atabrine.

(b) The skin level still rises when the blood level has almost returned to zero.

(c) A different scheme of dosage as suggested originally consisting of high initial doses brings about a more satisfactory curve of blood levels.

(d) A deposition of atabrine in the nail, detectable by jadelike fluorescence subsequent to its suppressive or therapeutic administration can be observed.

(e) A study of the nail fluorescence discloses whether and with what regularity atabrine has been taken. The presence or absence of zones of fluorescence in the nails was indicative of the administration or omission of atabrine.

This simple objective observation enables the examiner to establish the consistency with which the drug was taken under suppressive treatment.

We wish to take this opportunity to acknowledge our indebtedness to Rear Admiral Edward U. Reed, Medical Corps, United States Navy, District Medical Officer of the Third Naval District, for his stimulation and cooperation, and to the officers of the St. Albans Naval Hospital, for their kind collaboration.

REFERENCES

1. Chopra, R. N., and Roy, A. C.: Estimations of Minute Quantities of Atabrine in Blood, Indian M. Gaz. **70**: 504-505, 1935.
2. Weise, W.: Untersuchungen über die Resorption und Ausscheidungdes Atebrins; Methoden zur Bestimmung des Atebrins in Harn, Stuhl und Blut, Arch. f. Schiffs- u. Tropen-Hyg. **41**: 715-720, 1937.
3. Gentzkow, C. J.: Method for the Determination of Atabrine in the Blood, Am. J. Trop. Med. **18**: 149-156, 1938.
4. Brodie, B. B., and Udenfriend, S.: The Estimation of Atabrine in Biological Fluids and Tissues, J. Biol. Chem. **151**: 299-317, 1943.
5. Masen, J. M.: The Quantitative Determination of Atabrine in Blood and Urine, J. Biol. Chem. **148**: 529-539, 1943.
6. Auerbach, M. E., and Eckert, H. W.: Photofluorometric Determination of Atabrine, J. Biol. Chem. **154**: 597-603, 1944.
7. Lange, K., and Krewer, S. E.: The Dermofluorometer; an Instrument for Objective Measurement of Fluorescence of the Skin and Organs and Objective Determination of Circulation Time and Capillary Permeability, J. Lab. & Clin. Med. **28**: 1746-1750, 1943.
8. Lange, K., Matzner, M. J., and Krewer, S. E.: An Optical Fluorescence Comparator, J. Lab. and Clin. Med. **30**: 627-630, 1945.
9. Shannon, J. A., and others: Pharmacological Basis for Rational Use of Atabrine in the Treatment of Malaria, J. Pharmacol. & Exper. Therap. **81**: 307-330, 1944.
10. Lange, K., and Boyd, L. J.: The Use of the Fluorescein Method in Establishment of the Diagnosis and Prognosis of Peripheral Vascular Diseases, Arch. Int. Med. **74**: 175-184, 1944.

PYRIBENZAMINE (N'-PYRIDYL-N'-BENZYL-N-DIMETHYLETHYLENE-DIAMINE HCl), AN ANTAGONIST OF HISTAMINE

R. L. Mayer, H. W. Hays, D. Brousseau, D. Mathieson,
B. Rennick, and F. F. Yonkman
Summit, N. J.

PREVIOUS reports[1-4] have indicated briefly some of the antihistaminic potentialities of Pyribenzamine; this contribution extends some of the pharmacologic facts gained since these earlier publications and also presents data on toxicologic studies.

Toxicity.—

Acute: Acute toxicity was studied in various species of animals in which the drug was administered by various routes. In Table I data on the toxicity of pyribenzamine are given.

Death was usually preceded by marked excitability and convulsions; in some animals a secondary depression, varying from minutes to an hour or more, intervened between the period of excitation and death. In dogs which recovered from convulsions after 3 mg. or more intravenously, marked excitement and salivation gradually diminished during the next thirty to sixty minutes until complete recovery ensued.

Of special interest is the fact that castration of female rats rendered them less susceptible to the toxic and lethal effects of pyribenzamine; in fact, the LD_{50} of both sexes was then practically identical. Estrogen therapy of such castrate females was instituted with the anticipation that it might restore them to their previous greater vulnerability; the effect of such therapy is still a moot point.

Preliminary experiments demonstrated that pyribenzamine suspended in a beeswax*-sesame oil mixture according to Code and Varco[5] was well tolerated subcutaneously by guinea pigs in doses up to 50 mg. per kilogram; with higher doses an increasing number of deaths occurred from twelve to twenty-four hours after injection; with 150 mg. per kilogram the animals died within one and one-half hours after treatment.

Chronic: White rats of both sexes were given daily oral injections of pyribenzamine, 5 mg. per kilogram, and after five months there were no significant changes in erythrocytes, white cells and their differential distribution, hematocrit values, body weight, appetite, reproductive capacities, gastrointestinal functions and general appearance and comport.[6] These results agree with those of Koepf, Arbesman, and Munafo[7] concerning the relative safety of this compound after prolonged feeding.

Local: In some rats which survived acute toxicity studies, local necrosis of the skin developed over the site of subcutaneous injection. This was particularly

From the Departments of Bacteriology and Pharmacology, Division of Research, Ciba Pharmaceutical Products, Inc.

Received for publication, March 11, 1946.

*Ten per cent.

UNIVERSITY OF MICHIGAN LIBRARIES

TABLE I. LD_{50} OF PYRIBENZAMINE IN VARIOUS SPECIES ADMINISTERED BY DIFFERENT ROUTES

	NUMBER OF ANIMALS	ORAL (MG./KG.)	NUMBER OF ANIMALS	SUBCUTANEOUS (MG./KG.)	NUMBER OF ANIMALS	INTRAVENOUS (MG./KG.)
Mice	50	210	300	75	40	12
Rats						
Male	70	570	150	340	50	12
Female	70	515	90	225	35	12
Rabbits			32	33	36	9
Dogs					10	3-4*

*This does not represent LD_{50} but maximally tolerated dosages of pyribenzamine with recovery.

true in those animals receiving the larger amounts. This was not easily avoided because constant volume was desirable in order to minimize variability of absorption from injected areas of different size. Consequently, concentrations up to 30 per cent of pyribenzamine were required in order to establish the LD_{50} and such concentrations might well be necrotizing, either because of the general protoplasmic poisoning feature common to all substances known to have local anesthetic properties or because of vasoconstrictor effects which some locally-acting anesthetic agents may exert.

Intramuscular injections of pyribenzamine, 0.1 c.c. of 5 per cent, in the gastrocnemii of rats resulted in replacement of muscle cells by fibroblasts*; this result supports the suggestion that local necrosis may follow injudicious use of high concentrations of this drug hypodermically.

Oral in Man: Doses of 100 mg. in tablet form have been generally well tolerated by six human subjects, up to a daily total dosage of 500 mg. Minor complaints in two subjects, but not consistently present, included mild sedation in one and a sense of "pelvic heaviness" with mild nausea in another. It is probable, however, that this higher range of dosage would be more than adequate for the average clinical patient.[8]

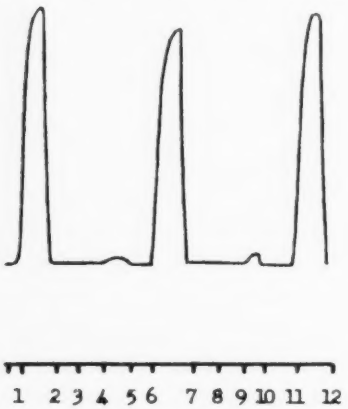

Fig. 1.

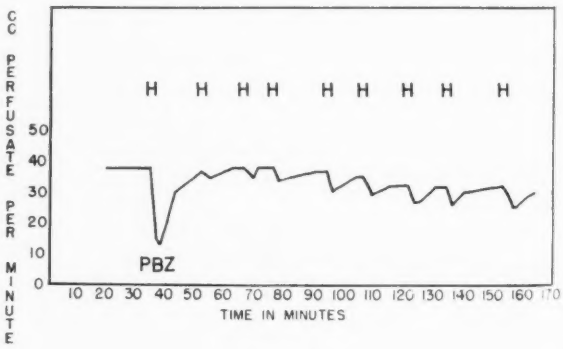

Fig. 2.

Fig. 1.—Intestinal strip from a guinea pig. Histamine, 1 μg, at *1, 4, 6, 9, 11;* pyribenzamine, 0.01 μg, at *3* and *8;* wash at *2, 5, 7, 10,* and *12.*

Fig. 2.—Studies of the perfused, excised guinea pig lung. The antihistaminic effect of pyribenzamine (N'-pyridyl-N'-benzyl-N-dimethylethylenediamine HCl), 0.025 mg., against histamine phosphate, 0.050 mg.

*We are indebted to Dr. James Leathem of the Department of Biology of Rutgers University, New Brunswick, N. J., for these pathologic findings.

Antihistaminic Properties (in Vitro).—

Intestine of Guinea Pig: Isolated segments of the jejunum responded to histamine by marked contraction. Much smaller doses of histamine are effective, but 1 μg was uniformly employed in order that some preliminary quantitative appraisal might be made of pyribenzamine in terms of antihistaminic potency. Although 0.02 μg. of pyribenzamine consistently inhibited the contractile response of the intestine to histamine, as little as 0.01 μg was frequently as effective (Fig. 1). Evaluation of the antihistaminic potency of pyribenzamine is now being determined quantitatively, with reference to the protection it affords against histamine hypotension in anesthetized dogs.

Uterus of Guinea Pig: Contraction of this muscle as produced by histamine, from 10 to 20 μg, was nullified by pyribenzamine, from 10 to 50 μg.

Bronchial Muscle of Guinea Pig: Excised lungs, perfused according to the modification of Langendorf's technique described by Tainter, Pedden, and James,[9] responded well to histamine by contraction of their bronchial muscle. The contractile effect of histamine, 50 μg was usually markedly arrested by pyribenzamine, 25 μg (Fig. 2), and recovery was not complete until after forty to ninety minutes or more.

CONCLUSIONS AND SUMMARY

The toxicity and antihistaminic actions of pyribenzamine are of such an order that extended investigations seem warranted.

REFERENCES

1. Mayer, R. L., Huttrer, C. P., Scholz, C. R.: Antihistaminic and Antianaphylactic Activity of Some α-pyridinoethylenediamines, Science **102**: 93, 1945; Antihistaminic and Antianaphylactic Activity in Vitro and in Vivo of Some α-aminopyridyl derivatives, Federation Proc. **4**: 129, 1945.
2. Mayer, R. L.: Antihistaminic Substances With Special Reference to Pyribenzamine, J. Allergy **17**: 153, 1946.
3. Yonkman, F. F., Hays, H. W., Rennick, B., Chess, D.: The Antihistaminic Action of N′-pyridyl-N′-benzyl-N-dimethylethylenediamine HCl (63C) in Relation to Salivation, Retraction of the Nictitating Membrane, Mydriasis, Lachrymation and Blood Pressure in Cats, Federation Proc. **4**: 143, 1945.
4. Rennick, B., Chess, D., Hays, H. W., Mathieson, D., Mayer, R. L., and Yonkman, F. F.: The Antianaphylactic and Antihistaminic Activity and Toxicity of N′-pyridyl-N′-benzyl-N-dimethylethylenediamine HCl, Federation Proc. **4**: 133, 1945.
5. Code, C. F., and Varco, R. L.: Prolonged Action of Histamine, Am. J. Physiol. **137**: 225, 1942.
6. Mathieson, D.: Personal communication.
7. Koepf, G., Arbesman, C., and Munafo, Claudia: Chronic Toxicity of Pyribenzamine, J. Allergy. In press.
8. Arbesman, C., Koepf, G., and Lenzner, A. F.: Clinical Studies With Pyribenzamine; a Preliminary Report, J. Allergy. In press.
9. Tainter, M. L., Pedden, J. R., and James, M.: Comparative Actions of Sympathomimetic Compounds: Bronchodilator Actions in Perfused Guinea Pig Lungs, J. Pharmacol. & Exper. Therap. **51**: 371, 1934.

UNIVERSITY OF MICHIGAN LIBRARIES

STUDIES ON THE TOXICITY OF SULFONAMIDE DRUGS

I. Porphyrin-Excretion by a Patient Treated With Sulfadiazine and Later With Sulfanilamide

Frank H. J. Figge, Ph.D., T. Nelson Carey, M.D., and
Glenn S. Weiland, Ph.D.
Baltimore, Md.

SOON after the introduction of sulfanilamide, it was noted that the administration of this drug gave rise to an increased excretion of urinary porphyrin in patients developing photosensitivity.[1] At about the same time, Rimington and Hemmings[2, 3] found an increase in the excretion of urinary and fecal coproporphyrin III and small quantities of coproporphyrin I in all patients and animals treated with sulfanilamide. In both of these investigations an attempt was made to relate some of the toxic symptoms of sulfanilamide to the increased production and excretion of porphyrin.

In preliminary attempts to verify these reports, the urine samples in hospital refrigerators were examined in a dark room in near ultraviolet light[4] to see if any of these were red fluorescent. Red fluorescent urines were not observed, but red or pink fluorescent precipitates were easily and frequently seen. The names and numbers on forty urine bottles containing red fluorescent precipitates were recorded over a period of weeks, and a check made on the hospital charts showed that the corresponding patients had received sulfonamide drugs in all but one instance. The exception was a patient with Hodgkin's disease who had received roentgen-ray therapy. This fluorescence examination of urines was carried out in 1942-1943, at which time sulfapyridine and sulfathiazole were popular. Many patients who were treated with sulfonamides could not be detected by this simple fluorescence test, probably because the urinary porphyrin excretion was not much higher than normal. Most of the urine samples with red fluorescent precipitates were from patients who had received sulfanilamide, but some sulfapyridine- and sulfathiazole-treated patients were detected by means of the red fluorescent urine sediment. This suggested the possibility that these abnormal porphyrins occurring in variable concentrations might be responsible for the bacteriostatic influence of the sulfonamide drugs. This idea had also been entertained and discarded by Rimington and Hemmings.[3] The reinvestigation of this problem confirmed his conclusions that the sulfonamide-induced porphyrins probably have no connection with the therapeutic effect but rather appear to be linked with photosensitivity, anuria, neurologic symptoms, and perhaps other toxic effects.

From the Departments of Anatomy, Medicine, and Biochemistry, University of Maryland School of Medicine.

Aided by grants from The Anna Fuller Fund, The International Cancer Research Foundation, and The Bressler Alumni Research Fund.

We wish to acknowledge and express appreciation for the assistance and cooperation of Dr. Louis P. Manganiello in the initial phase of this work.

Received for publication, March 8, 1946.

One of the questions which arose was this: Do the various sulfonamides give rise to the same porphyrin or are specific porphyrins produced by each sulfonamide? A definite answer to this question might indicate whether the excess porphyrins were involved in the specific therapeutic effects or in the toxic effects of sulfonamides. The recent note on the inhibition of bacterial growth by coproporphyrin, hematoporphyrin, and deuteroporphyrin was of interest in this connection.[5]

In this paper it will be shown that different sulfonamides always gave rise to excess amounts of only coproporphyrin III in the same individual.

Because of the laborious procedures involved in extraction and identification of porphyrins in daily collections of urine, it was decided to investigate this problem carefully in one individual who was treated with various sulfonamides at different times. The patient was a 42-year-old white male farmer who came to the hospital April 10, 1943, with empyema on the right side. He was treated for five days with sulfadiazine (6 Gm. per day) (see Fig. 1). On

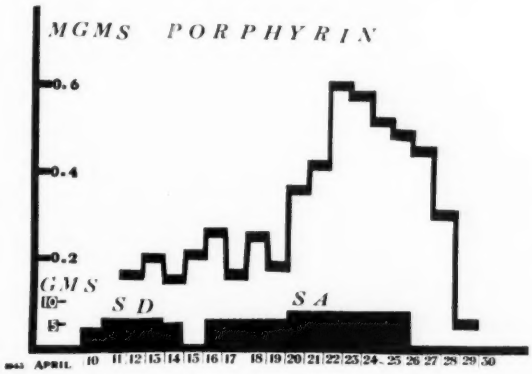

Fig. 1.—Coproporphyrin III excretion after treatment with sulfadiazine and sulfanilamide. Chart, showing the daily excretion of coproporphyrin III with varying dosage of sulfadiazine followed by sulfanilamide. Note the great increase observed when the sulfanilamide was increased to 8 Gm. per day.

the sixth day no sulfonamide drugs were administered. Sulfanilamide was then started. For the next four days the patient received 6 Gm. of sulfanilamide per day, at which time the dosage was increased to 8 Gm. per day and maintained at this level for six more days (see Fig. 1). Thus a total of 27 Gm. of sulfadiazine and 74 Gm. of sulfanilamide were given in fifteen days. During this time the urine was collected over twenty-four hour periods for the determination of the urinary porphyrins. Dobriner's methods of extraction of the urine were employed.[6] The porphyrins isolated were identified by the following methods:

1. The determination of absorption bands by means of a large spectroscope with a long wave length scale
2. The determination of the HCl number
3. The solubility in 20 per cent NaOH, chloroform, and ether
4. The melting point of the methyl ester

UNIVERSITY OF MICHIGAN LIBRARIES

The quantity of porphyrin excreted daily was determined by measuring the absorption of a beam of light (4050 A°) isolated by a set of Corning filter glasses,* with a very sensitive colorimeter.† It was found possible to determine accurately the concentration of standard solutions of various porphyrins containing from 0.1 to 1.0 μg per cubic centimeter. Lower concentrations may be determined by using the same instrument as a fluorescence meter, but because this technique involves the careful control of a number of factors (temperature, purity, and HCl concentration), this method has been found to be tedious and less accurate than the colorimetric method which uses light with a wave length of from 4000 to 4100 A°.

The quantities of porphyrin excreted daily have been depicted graphically with the dosage of sulfonamides shown in Fig. 1. The collection of urine was not started until the third day of treatment with sulfadiazine. Four daily collections were extracted which contained enough sulfadiazine-induced porphyrin to identify as coproporphyrin III. The urine collected during the eleven days that the patient was treated with sulfanilamide contained far greater amounts of coproporphyrin III. The identification was carried out in the following manner: An attempt was made to fractionate the porphyrin obtained by extraction from ether with increasing concentrations of hydrochloric acid. Most of the porphyrin was extracted with 0.1 per cent hydrochloric acid. The remaining porphyrin appeared in the 0.6 per cent fraction. However, when both fractions were passed back into ether, they had identical absorption spectra which coincided with a sample of synthetic coproporphyrin I and with that recorded for coproporphyrin by other investigators.[7] When the various fractions of the porphyrin were extracted from the ether with 25 per cent hydrochloric acid, the absorption spectra of all fractions were identical. The position of the middle of the absorption bands on the wave length scale were as follows (stated in millimicrons): in ether, 623.8, 597.2 577.2, 568.2, 528.4, 495.0; in 25 per cent hydrochloric acid, the bands were located at 593.2, 572.6, 550.3. The porphyrin extracted from the urine of the patient during the time that sulfanilamide was administered had the same absorption bands and solubility characteristics as that extracted from the urine during the period of sulfadiazine treatment. None of the porphyrin extracted was soluble in chloroform, but it dissolved in 20 per cent sodium hydroxide. To see if this was coproporphyrin I or III, the various porphyrin fractions were then pooled and converted into the methyl ester to determine the melting point. The absorption spectrum of the methyl ester in ether was determined on a spectrophotometer. The bands were located at 625.9, 597.5, 570.0, 528.0, 494.0. The melting point of the methyl ester crystals was from 145.2 to 147° C. When remelted the crystals melted at from 165 to 168° C. Therefore, we concluded that the porphyrin was coproporphyrin III.

The fact that the same porphyrin is excreted when one individual is treated with different sulfonamides should have been expected on the basis of the investigations of Rimington and Hemmings.[3] They tested a number of

*Corning 3060, 430, 385.
†Lumetron 402 EF.

compounds resembling sulfanilamide or prontosil and found the porphyrin-inducing property related to the phenylamine group. Aniline and similar substances were found to induce porphyrinuria. Since the phenylamine group is essential for therapeutic activity, it is not modified in most of the sulfonamides. They should, according to Rimington's hypothesis, all undergo oxidation in vivo and in turn oxidize hemoglobin to methemoglobin which is degraded perhaps by way of hematin to coproporphyrin III instead of bilirubin. If this process is responsible for the toxic effects of sulfonamides, then all the sulfonamides commonly used should present toxic symptoms of the same type and degree. Such is, however, not the case.

A survey of the literature revealed that, in addition to Brunsting[1] and Rimington and Hemmings,[3] other investigators had reported porphyrinuria resulting from sulfonamide treatment. Long and Bliss,[8] in a discussion of the toxic manifestations of sulfanilamide, stated that approximately 3 per cent of the patients exhibited porphyrinuria. Wien[9] confirmed Rimington and Hemming's observation that sulfanilamide administration causes porphyrinuria in the human subject and in rats, but he claimed that sulfapyridine did not significantly alter the rate of porphyrin excretion in rats. The values which Wien gave as normal for rats ranged from 24 to 48 μg per rat per day. The range found for sulfapyridine-treated rats was from 36 to 84 μg. The rats given sulfanilamide excreted from 90 to 260 μg daily. During this same year other investigators[10] found normal urinary porphyrin values in several patients who had taken sulfanilamide, and one of these was light sensitive. The methods used to extract and determine the porphyrin in this case are open to question. Small quantities of urine were extracted, and the relatively crude and impure porphyrin extracts were roughly estimated by visual fluorometry. More recently, Schreus and Kreig[11] determined the porphyrin excretion in thirteen patients with either gonorrhea or pyoderma treated with various sulfonamides. Eight of the thirteen showed slight increases in porphyrin excretion. This gives the impression that a marked increase such as we have observed (from 200 to 600 μg per day) occurs only sporadically in sulfonamide-treated patients. This is doubtlessly true, and the amount of porphyrin excreted in any given case is probably determined by the type of sulfonamide given, the dosage, the individual susceptibility or condition of the patient, and perhaps the disease.

The possibility that some of the severe toxic reactions which occur infrequently are limited to those patients who respond to sulfonamides by producing and excreting relatively large amounts of porphyrin deserves further investigation.

SUMMARY AND CONCLUSIONS

The fact that sulfonamide therapy frequently gives rise to a mild and variable porphyrinuria has been confirmed by semiquantitative observations on forty patients. In a careful study of the urinary porphyrins of one of these, it was observed that the administration of either sulfadiazine or sulfanilamide to the same patient at different times gave rise to the excretion of

UNIVERSITY OF MICHIGAN LIBRARIES

abnormal amounts of the same kind and type of porphyrin, coproporphyrin III. The amount of porphyrin excreted during sulfadiazine treatment was less than when a similar dose of sulfanilamide was administered. The amounts of porphyrin excreted in this case paralleled the relative toxicity of the two drugs. Since aniline, lead salts, sulfonal, and other similar compounds give rise to porphyrinuria, but presumably have no bacteriostatic action, the coproporphyrin III is probably one of the coincidental by-products of sulfonamide action.

REFERENCES

1. Brunsting, L. A.: Sulfanilamide Dermatitis: Question of Relation to Photosensitivity, Proc. Staff Meet., Mayo Clin. **12**: 614-616, 1937.
2. Rimington, C.: Porphyrinuria Following Sulphanilamide: Sulphanilamide Dermatitis, Lancet **1**: 770-776, 1938.
3. Rimington, C., and Hemmings, A. W.: Porphyrinuric Action of Drugs Related to Sulphanilamide. Comparison With Reported Toxicity, Therapeutic Efficiency and Causation of Methaemoglobinaemia. Definition of the Structure Responsible for Porphyrinuric action, Biochem. J. **33**: 960-977, 1939.
4. Figge, F. H. J.: Near-Ultraviolet Rays and Fluorescence Phenomena as Aids to Discovery and Diagnosis in Medicine, Bull. School Med. Univ. Maryland **26**: 165-176, 1942.
5. Granick, S., and Gilder, H.: The Structure, Function and Inhibitory Action of Porphyrins, Science **101**: 540, 1945.
6. Dobriner, K.: Urinary Porphyrins in Disease, J. Biol. Chem. **113**: 1-10, 1936.
7. Fischer, H., and Orth, H.: Die Chemie des Pyrrols, vol. 2, 1st half, Leipzig, 1937, Akademische Verlagsgesellschaft m. b. H. Druck von Paul Dunnhaupt, Köthen, pp. 395-498.
8. Long, P. H., and Bliss, E. A.: Toxic Manifestation of Sulfanilamide, Ann. Surg. **108**: 808-812, 1938.
9. Wien, R.: The Toxicity of 2-(p-aminobenzenesulphonamido) Pyridine, Quart. J. Pharm. & Pharmacol. **11**: 217-224, 1938.
10. McFarland, A. R., and Strain, W. H.: Significance of Porphyrin Content of Urine in Dermatoses Associated With Sensitivity to Light, Arch. Dermat. & Syph. **38**: 727-736, 1938.
11. Schreus, H. T., and Kreig, J.: Porphyrin Formation in Sulfonamide Therapy, Arch. Dermat. **182**: 421-427, 1941.

OBSERVATIONS ON THE ACTIVITY IN VITRO OF SUCCINYLSULFATHIAZOLE AND PHTHALYLSULFATHIAZOLE

H. J. Florestano, Ph.D., and J. F. Kennedy*
Indianapolis, Ind.

THE recent studies by Poth and co-workers[1, 2] on a series of N^4-dicarboxylic acid-substituted sulfonamides synthesized by Moore and Miller[3] resulted in the development of two new sulfonamides, succinylsulfathiazole and phthalylsulfathiazole, which have found extensive use as intestinal antiseptics. Theoretically, these two N^4-acyl derivatives of sulfathiazole should have no activity in vitro. However, the few reports in the literature regarding this property have been conflicting. Kirby and Rantz[4] found that succinylsulfathiazole had practically no activity in vitro. Poth and associates[1] reported slight bacteriostatic activity for succinylsulfathiazole against *Escherichia coli* in vitro but thought that the activity shown might have been due to a small amount of free sulfathiazole present in the sample studied. In a later report, however, Poth and Ross[5] used data obtained in vitro with succinylsulfathiazole in a comparison of results obtained with a number of carboxylic acid derivatives of sulfathiazole. Brewer[6] was also able to demonstrate activity in vitro for succinylsulfathiazole. With *Shigella sonnei* as the test organism, the activity was found to be directly proportional to the amount of drug present and inversely proportional to the number of organisms. In comparison, sulfaguanidine had no activity against the same organism. Schweinberg and Yetwin[7] observed that phthalylsulfathiazole had a better bactericidal action than sulfathiazole against *E. typhosa* and *Shigella paradysenteriae* (var. Flexner), while sulfathiazole was the more effective of the two drugs against *E. coli*, *Shigella dysenteriae* (Shiga) and the paradysenteriae strain of Hiss.

Evidence is presented here in an attempt to show that demonstrable activity in vitro for both succinylsulfathiazole and phthalylsulfathiazole is due not to each drug per se but apparently to free sulfathiazole which not only may be present as a contaminant in the original material but can be augmented in concentration as a result of decomposition of the conjugated sulfathiazole during preparation for testing.

EXPERIMENTAL

As a preliminary to the studies in vitro, a number of solutions of succinylsulfathiazole and phthalylsulfathiazole† were prepared at different concentrations in distilled water buffered at pH 7.0.‡ The drugs were dissolved at room temperature and then analyzed for free diazotizable substance after the method

From the Research Department, Pitman-Moore Co., Division of Allied Laboratories, Inc.
Received for publication, April 27, 1946.

*Present address: Wallace Laboratories, Inc., New Brunswick, N. J.

†Samples of the drugs were purchased from the open market.

‡The buffered water (pH 7.0) was prepared by the addition of 6.805 Gm. of KH_2PO_4 and 29.63 c.c. of 1 N NaOH to distilled water to make 1 liter of solution.

UNIVERSITY OF MICHIGAN LIBRARIES

of Bratton and Marshall.[5] Aliquots of the solutions were autoclaved at 15 pounds pressure for varying periods of time, and diazotization was again performed on the respective solutions. Because of the extreme rapidity with which phthalylsulfathiazole hydrolyzes in acid solution, it was necessary to modify the diazotization procedure somewhat in order to obtain reproducible results. Typical findings from these analyses are shown in Table I. The results indicated that the samples of succinylsulfathiazole and phthalylsulfathiazole studied were contaminated with a substance capable of diazotization. It was also observed that the amount of this substance could be increased by heating, presumably as a result of decomposition of the original N^4-acylated sulfathiazole derivatives.

TABLE I. FREE DIAZOTIZABLE SUBSTANCE IN SOLUTIONS OF SUCCINYLSULFATHIAZOLE AND PHTHALYLSULFATHIAZOLE BEFORE AND AFTER AUTOCLAVING

CONCENTRATION OF DRUG BY WEIGHT (MG. %)	TREATMENT	DIAZOTIZABLE SUBSTANCE* IN SUCCINYLSULFATHIAZOLE		DIAZOTIZABLE SUBSTANCE* IN PHTHALYLSULFATHIAZOLE	
		CONCENTRATION (MG. %)	PER CENT OF TOTAL WEIGHT	CONCENTRATION (MG. %)	PER CENT OF TOTAL WEIGHT
16	Unheated			0.19	1.19
	5 min. heat			1.26	7.88
	20 min. heat			2.50	15.63
32	Unheated	0.10	0.31	0.33	1.03
	5 min. heat	0.18	0.56	2.74	8.56
	20 min. heat	0.30	0.94	5.55	17.34
64	Unheated	0.19	0.30	0.62	0.97
	5 min. heat	0.40	0.63	5.15	8.10
	20 min. heat	0.64	1.00	9.80	15.31
128	Unheated	0.31	0.24		
	5 min. heat	0.62	0.48		
	20 min. heat	1.02	0.80		

*Calculated as free sulfathiazole.

TABLE II. HYDROGEN ION CONCENTRATIONS OF SOLUTIONS OF SUCCINYLSULFATHIAZOLE AND PHTHALYLSULFATHIAZOLE PREPARED IN WATER BUFFERED AT PH 7.0

CONCENTRATION OF SOLUTION (MG. %)	DRUG*	HYDROGEN-ION CONCENTRATION (pH)		
		INITIAL	AFTER 5 MIN. HEAT	AFTER 20 MIN. HEAT
16	PST	7.00	7.00	7.00
	SST	6.98	6.98	6.98
32	PST	6.97	6.97	6.97
	SST	6.93	6.93	6.93
64	PST	6.93	6.93	6.93
128	SST	6.86	6.86	6.86

*PST, Phthalylsulfathiazole; SST, succinylsulfathiazole.

As a check on the buffering capacity of the diluent used for the various solutions, the pH of each solution was determined immediately upon dissolution of drug and after autoclaving. No marked change from the original pH of the buffered water was indicated when solutions of drug as high as 128 mg. per 100 c.c. were prepared. The initial hydrogen ion concentrations of all solutions remained unaltered when heated at 15 pounds pressure for twenty minutes (Table II).

On the assumption that the varying degrees of diazotization were due to the presence of free sulfathiazole, the following procedure was used to test the hypothesis.

Solutions of succinylsulfathiazole and phthalylsulfathiazole were prepared in a synthetic medium consisting of asparagine, glucose, and inorganic salts.[9] Concentrations for the various tests ranged from 32 to 512 mg. per cent by weight. All solutions were made up at room temperature, and the amount of free diazotizable substance was determined for each. An aliquot of each solution was then sterilized by Seitz filtration while a second and third portion were autoclaved at 15 pounds pressure for five and twenty minutes, respectively. The Bratton and Marshall reaction was again carried out on the Seitz filtrates and heated portions. Solutions of sulfathiazole were next prepared in the synthetic medium to contain concentrations of the drug comparable to the respective amounts of free diazotizable substance (calculated as sulfathiazole) in each of the variously treated solutions of succinylsulfathiazole and phthalylsulfathiazole. All sulfathiazole solutions were checked by the color reaction before use, and each of these corresponding to the respective solutions of the N^4-acylated derivatives was similarly treated; that is, one portion was passed through a Seitz filter and a second was autoclaved for five minutes, a third for twenty minutes. This procedure was followed throughout, even though filtration and heating had no apparent effect whatever on the initial concentrations of sulfathiazole.

The comparison in vitro of the conjugated derivatives of sulfathiazole with free sulfathiazole was performed by serially diluting, aseptically, each initial drug solution by halves in plain sterile synthetic medium and inoculating with *E. coli*. Each tube contained a final volume of 5.0 c.c. of medium plus drug at the various dilutions, and 0.1 c.c. of a diluted eighteen-hour culture was added so that the initial number of organisms averaged about 500 per cubic centimeter. The tubes were incubated at 37° C. and examined for visible growth at twenty-four hour intervals.

RESULTS

It was apparent from the data obtained that succinylsulfathiazole and phthalylsulfathiazole exerted inhibitory action against *E. coli* under the conditions of the test. However, when free sulfathiazole was tested at concentrations corresponding to those of the free diazotizable substance present in the N^4-acylated sulfathiazole solutions, identical end points resulted. For example, a concentration of 32 mg. per 100 c.c. of succinylsulfathiazole (Seitz-filtered) showed complete stasis, while partial inhibition of *E. coli* was affected at 16 mg. per 100 c.c. The amount of free diazotizable substance at each of these concentrations of succinylsulfathiazole was equivalent to 0.08 and 0.04 mg. of sulfathiazole per 100 c.c., respectively, at which concentrations free sulfathiazole produced the same results (Table III). When an aliquot of the same initial solution of succinylsulfathiazole was autoclaved for twenty minutes, complete stasis occurred at a concentration of 4.0 mg. per 100 c.c., at which level 0.06 mg. per 100 c.c. was present as free diazotizable substance. A corresponding amount of free sulfathiazole (0.06 mg. per 100 c.c.) likewise showed complete

UNIVERSITY OF MICHIGAN LIBRARIES

TABLE III. BACTERIOSTATIC ACTION OF SUCCINYLSULFATHIAZOLE AND SULFATHIAZOLE

	SUCCINYLSULFATHIAZOLE						SULFATHIAZOLE				
	CONCENTRATION BY WEIGHT	CONCENTRATION OF FREE DIAZOTIZABLE SUBSTANCE*	TIME (HR.)				CONCENTRATION	TIME (HR.)			
TREATMENT	(MG. %)	(MG. %)	24	48	72	144	(MG. %)	24	48	72	144
Seitz-filtered	64	0.16	0	0	0	0	0.16	0	0	0	0
	32	0.08	0	0	0	0	0.08	0	0	0	0
	16	0.04	0	0	+	+++	0.04	0	0	+	+++
	8	0.02	+	++++	++++	++++	0.02	+	++++	++++	++++
	4	0.01	++++	++++	++++	++++	0.01	++++	++++	++++	++++
Autoclaved (20 min.)	64	1.00	0	0	0	0	1.00	0	0	0	0
	4	0.06	0	0	0	0	0.06	0	0	0	0
	2	0.03	0	+	+	+++	0.03	0	+	+	+++
	1	0.015	+	++++	++++	++++	0.015	+	++++	++++	++++
	0.5	0.007	++++	++++	++++	++++	0.007	++++	++++	++++	++++

*Calculated as free sulfathiazole.
0, No growth; ++++, maximum growth.

TABLE IV. BACTERIOSTATIC ACTION OF PHTHALYLSULFATHIAZOLE AND SULFATHIAZOLE

	PHTHALYLSULFATHIAZOLE						SULFATHIAZOLE				
	CONCENTRATION BY WEIGHT	CONCENTRATION OF FREE DIAZOTIZABLE SUBSTANCE*	TIME (HR.)				CONCENTRATION	TIME (HR.)			
TREATMENT	(MG. %)	(MG. %)	24	48	72	144	(MG. %)	24	48	72	144
Seitz-filtered	32	0.72	0	0	0	0	0.72	0	0	0	0
	4	0.09	0	0	0	0	0.09	0	0	0	0
	2	0.045	0	++	+++	+++	0.045	0	++	+++	+++
	1	0.022	+++	++++	++++	++++	0.022	+++	++++	++++	++++
	0.5	0.011	++++	++++	++++	++++	0.011	++++	++++	++++	++++
Autoclaved (5 min.)	32	7.80	0	0	0	0	7.80	0	0	0	0
	0.25	0.06	0	0	0	0	0.06	0	0	0	0
	0.125	0.03	0	++	+++	++++	0.03	0	++	+++	++++
	0.062	0.015	++	++++	++++	++++	0.015	++	++++	++++	++++
	0.031	0.007	++++	++++	++++	++++	0.007	++++	++++	++++	++++

*Calculated as free sulfathiazole.
0, No growth; ++++, maximum growth.

inhibition of growth of the test organism. The protocol of an experiment illustrating a similar comparison of activity with phthalylsulfathiazole and sulfathiazole is given in Table IV.

DISCUSSION

It is believed that the apparent activity in vitro shown by succinylsulfathiazole and phthalylsulfathiazole has been identified with that of free sulfathiazole. Hydrolysis of either of these N^4-acylated compounds would yield sulfathiazole and the respective dibasic acid. The possibility of any antibacterial activity due to either succinic acid or phthalic acid alone was ruled out, however, since tests in vitro showed them to have no effect on *E. coli* in concentrations much higher even than those expected through decomposition.

The exact bearing that these findings may have in relation to the in vivo mode of action of succinylsulfathiazole and phthalylsulfathiazole cannot be stated conclusively at this time. Poth and associates[1] originally believed that these drugs might owe their antibacterial activity to free sulfathiazole liberated by hydrolysis of the acylated derivatives. It was concluded from later work,[10, 11] however, that this alone could not fully explain their action and that a large portion of the activity of succinylsulfathiazole and phthalylsulfathiazole must consequently be attributed to the conjugated molecule.

The bacteriostatic effectiveness of sulfanilamide derivatives has been associated with the free amino group situated in the para position of the benzene nucleus. Activity may be destroyed through conjugation in the body or by chemical substitution otherwise. This fact becomes of importance in the study of the behavior in vitro of certain derivatives of the parent sulfonamides, especially N^4-acylated compounds. Caution should be exercised, therefore, in ascertaining first of all the purity of such compounds and secondly whether the substances are stable enough to withstand the various steps involved in their preparation for assay.

SUMMARY

1. Data are presented showing that succinylsulfathiazole and phthalylsulfathiazole may contain a diazotizable substance as an impurity. The amount of this substance can be progressively increased by prolonged heating of the N^4-acylated derivatives of sulfathiazole in solution.

2. It is concluded that the impurity is sulfathiazole and that its presence accounts for any in vitro activity shown by the conjugated sulfathiazoles.

REFERENCES

1. Poth, E. J., Knotts, F. L., Lee, J. T., and Inui, F.: Bacteriostatic Properties of Sulfanilamide and Some of Its Derivatives. I. Succinylsulfathiazole, a New Chemotherapeutic Agent Locally Active in the Gastrointestinal Tract, Arch. Surg. **44**: 187, 1942.
2. Poth, E. J., and Ross, C. A.: Bacteriostatic Properties of Sulfanilamide and Some of Its Derivatives. II. Phthalylsulfathiazole, a New Chemotherapeutic Agent Locally Active in the Gastroenteric Tract, Texas Rep. Biol. & Med. **1**: 345, 1943.
3. Moore, M. L., and Miller, C. S.: Dicarboxylic Acid Derivatives of Sulfonamides, J. Am. Chem. Soc. **64**: 1572, 1942.
4. Kirby, W. M. M., and Rantz, L. A.: The Treatment of Typhoid and Dysentery Carriers With Succinylsulfathiazole, J. A. M. A. **119**: 615, 1942.

UNIVERSITY OF MICHIGAN LIBRARIES

5. Poth, E. J., and Ross, C. A.: Bacteriostatic Properties of Sulfanilamide and Some of Its Derivatives. III. The Carboxy-Sulfathiazoles as Chemotherapeutic Agents Active in the Gastrointestinal Tract, J. Lab. & Clin. Med. **30**: 843, 1945.
6. Brewer, A. E.: Sonne Dysentery Carriers Treated With Succinylsulfathiazole, Lancet **2**: 471, 1944.
7. Schweinberg, F. B., and Yetwin, I. J.: In Vitro Action of Sulfamerazine, Phthalylsulfadiazine, Phthalylsulfamerazine and Phthalylsulfathiazole on Enteric Pathogens, New England J. Med. **230**: 510, 1944.
8. Bratton, A. C., and Marshall, E. K., Jr.: A New Coupling Component for Sulfanilamide Determination, J. Biol. Chem. **128**: 537, 1939.
9. MacCleod, C. M.: The Inhibition of the Bacteriostatic Action of Sulfonamide Drugs by Substances of Animal and Bacterial Origin, J. Exper. Med. **72**: 217, 1940.
10. Poth, E. J., and Ross, C. A.: The Clinical Use of Phthalylsulfathiazole, J. Lab. & Clin. Med. **29**: 785, 1944.
11. Poth, E. J., and Ross, C. A.: Studies on Rates of Hydrolysis of N^4 Dibasic Acid Substituted Sulfonamides, Proc. Soc. Exper. Biol. & Med. **57**: 322, 1944.

FATE OF SODIUM RICINOLEATE AFTER ORAL ADMINISTRATION TO WHITE RATS

E. L. MacQuiddy, M.D.,* and Elizabeth Pinkerton, Ph.D.,* Omaha, Neb., and Hartmann Goetze, B.S.,† and Fred W. Oberst, Ph.D.,† Cincinnati, Ohio

MUCH has been published with regard to the properties of sodium ricinoleate, but little is known of its action in the intestinal tract when it is used in the treatment of certain chronic intestinal disorders. It is believed that the therapeutic action may be due to detoxication of many intestinal toxins or to alteration of the intestinal flora.[1-4] Solutions of sodium ricinoleate are capable of neutralizing various bacterial toxins, phytotoxins, and cobra venom in vitro and possibly in the living animal.[2, 5-9] Some of the detoxicated toxins when injected into various animals have produced immunity against many times the lethal dose of the respective toxins. Consideration has also been given to its bactericidal or bacteriostatic action on many organisms. Kolmer[9] found sodium ricinoleate to have a low bactericidal activity in the test tube.

As yet there are few facts upon which to base an adequate explanation of the therapeutic action of sodium ricinoleate in the intestinal tract.

In an earlier study[10] no evidence was obtained that ricinoleate soaps or castor oil are absorbed from the intestinal tract, as determined by chylomicron count or by absorption of sudan IV. Paul and McCay[11] fed guinea pigs a diet containing 6 per cent ricinoleic acid and found from 91 to 93 per cent utilized as determined by fecal analyses. Castor oil, when present in the diet at a level af 6 per cent, was utilized to the extent of 92 per cent in rabbits and 99 per cent in sheep. They believe that melting point rather than degree of saturation is the determining factor in the utilization of fats in guinea pigs.

Recently Stewart and Sinclair[12] found the amount of fats excreted in rats on a diet containing 48.4 per cent castor oil to be about 2 per cent of the intake. They concluded that ricinoleic acid is readily metabolized, as there was no increase in the acetyl number of fecal fat over the control values and no evidence of its presence in the phospholipids of the small intestine, liver and muscle, or glycerides of the liver. Of the fatty acids of depot fat, about 7 per cent was ricinoleic acid.

In the present study sodium ricinoleate was either fed in a diet or administered by stomach tube to white rats, and the amounts absorbed from the gastrointestinal tract or excreted in feces were determined.

EXPERIMENTAL STUDY

The work involved either feeding or administering by stomach tube sodium ricinoleate‡ to white rats. The extraction of fats and fatty acids was made from gastrointestinal contents and feces, with subsequent separation into petroleum

Received for publication, April 4, 1946.

*From the University of Nebraska, School of Medicine.

†From the Department of Biochemistry, Research Laboratories, The Wm. S. Merrell Company.

‡Soricin, The Wm. S. Merrell Brand of Sodium Ricinoleate.

UNIVERSITY OF MICHIGAN LIBRARIES

ether (from 40 to 60° C.) and ethyl ether fractions. The fatty acid of sodium ricinoleate has a low solubility in petroleum ether but is completely soluble in ethyl ether. Its high acetyl number is useful for identification. The presence of ricinoleic acid was based on the total amount of fats and fatty acids extracted, amounts extractable by petroleum ether followed by re-extractions with ethyl ether and by acetyl numbers of the two fractions.

Procedure for Carrying Out Excretion Studies.—Sodium ricinoleate was administered to white rats either by stomach tube or by incorporating it into a stock diet.* Those animals receiving it by stomach tube were given 0.4 Gm., and the feces were collected for forty-eight hours. Those animals receiving it in their diet consumed approximately 0.5 Gm. daily for a period of two months. The feces were collected during the entire period and an aliquot portion used for the determinations.

Feces were collected also from control animals not receiving sodium ricinoleate. Recovery tests of sodium ricinoleate added to feces also were made.

Methods for Preparation, Extraction, and Testing of Materials.—All samples of feces were dried at 100° C. for twelve hours, weighed, and then powdered. A suitable aliquot was transferred to a separatory funnel containing 25 c.c. of distilled water. The mixture was made acid with 10 per cent hydrochloric acid, and the total fats and fatty acids were extracted by shaking with 5 c.c. portions of chloroform. The extracts were filtered successively through filter paper into a tared flask. The combined extract was evaporated to dryness on a steam bath under a current of air. The weighed residue was dissolved in 20 c.c. of absolute ethanol and transferred to a separatory funnel. About 10 c.c. of water was added, after which it was washed three times with 30 c.c. portions of petroleum ether. The petroleum ether fraction was washed twice with 15 c.c. portions of 50 per cent ethanol, which was transferred back to the alcoholic solution. The petroleum ether fraction was evaporated to dryness in a tared flask and weighed. Fifty cubic centimeters of ethyl ether and enough distilled water were added to the alcoholic solution to cause separation. The ethyl ether fraction was drawn off, and the aqueous alcoholic mixture was extracted three more times with ethyl ether. The ethyl ether extractions were combined in a tared flask, evaporated to dryness, and weighed.

The acetyl value of each fraction was determined by the method of Roberts and Schuette.[13]

Absorption Studies of Sodium Ricinoleate From Gastrointestinal Tract.—Acute studies were made on the amount of fats and fatty acids found in various segments of the gastrointestinal tract of rats twenty-four hours after oral administration of a large dose of sodium ricinoleate. Eight white rats, all weighing approximately 220 grams each, were used in the experiment. They were without food for from sixteen to twenty-four hours. Four were administered 2 c.c. of distilled water by means of a stomach tube and were used as controls. The other four animals received 400 mg. of sodium ricinoleate in 2 c.c. of distilled water. The feces were collected from each animal. All animals were sac-

*"Harlan Special Rat Ration" obtained from Harlan Small Animal Industry, Cumberland, Ind. The fat content of this diet is 4.4 per cent.

rificed twenty-four hours after gastric intubation, and the stomach and intestines were removed. The contents of the stomach and small intestine were washed into a separatory funnel by means of a stream of hot water. The contents of the large intestine and the feces were transferred to another separatory funnel. The aqueous mixtures were acidified with hydrochloric acid and extracted five times with 25 c.c. portions of chloroform. These were filtered successively through chloroform-wetted cotton into a tared evaporating flask. The filtrates were evaporated to dryness on a steam bath and placed in a vacuum desiccator overnight. The flasks were weighed again to determine the total fat by difference.

Similar acute studies were made on another group of rats to determine the amount of lipids remaining in the stomach and various segments of the intestinal tract, one-half, three, six, sixteen, and twenty-four hours after administration of 400 mg. of sodium ricinoleate in 2 c.c. of water by stomach tube. The animals, which weighed approximately 200 grams each, were without food for from sixteen to twenty-four hours before the test. Animal charcoal was given with the sodium ricinoleate to show the distance it had traveled down the intestinal tract at the time each animal was killed.

Three control animals received the animal charcoal in 2 c.c. of water but no sodium ricinoleate. One of these rats was killed one-half hour, another three hours, and the third six hours later. The contents of the stomach, of the upper, and of the lower half of the small intestine of all animals were removed and assayed for the amounts of lipids by the method just described.

RESULTS

The results of the excretion studies are shown in Table I. Under the condition of the experiment approximately 25 per cent of ricinoleic acid is extracted by petroleum ether and 75 per cent by ethyl ether. Under similar experimental conditions the total fat from the control feces was about equally divided between the petroleum ether and ethyl ether fractions. When 300 mg. sodium ricinoleate were added to 2 Gm. of control feces, the total fats and fatty acids recovered were re-extracted with petroleum ether and then with ethyl ether. The amounts of material dissolved in the two solvents were 33 and 65 per cent, respectively, indicating by its solubility that ricinoleic acid was present. The acetyl value for each fraction was considerably higher than that of the respective control sample.

TABLE I. PARTITION OF TOTAL LIPIDS BETWEEN PETROLEUM ETHER AND ETHYL ETHER, TOGETHER WITH ACETYL NUMBERS FOR EACH FRACTION

MATERIAL	NUMBER OF TESTS	TOTAL LIPIDS FOUND IN MATERIAL (PER CENT)	PETROLEUM ETHER FRACTION		ETHYL ETHER FRACTION	
			LIPIDS (PER CENT OF TOTAL)	ACETYL NUMBER	LIPIDS (PER CENT OF TOTAL)	ACETYL NUMBER
Sodium ricinoleate (300 mg.)	2	84.4	24.1	242	74.8	242
Control feces (2 Gm.)	2	6.6	53.4	141	47.6	156
Sodium ricinoleate (300 mg.) added to control feces (2 Gm.)	2	16.3	32.9	218	64.5	219
Feces from animals receiving 0.5 Gm. sodium ricinoleate daily	4	6.0	59.9	128	36.8	142

UNIVERSITY OF MICHIGAN LIBRARIES

Values obtained after the administration of a single dose of sodium ricinoleate or after the daily feeding of 0.5 Gm. of the material for two months are quite similar to those obtained with control feces and indicate the absence of ricinoleic acid. This would suggest that sodium ricinoleate either is absorbed from the intestinal tract or is so altered chemically as not to be extracted by the technique used.

In an experiment in which 400 mg. of sodium rinicoleate were administered orally and the rats killed twenty-four hours later, there was no indication that an appreciable amount of the administered substance was present in the gastrointestinal tract. In the control group the average weight of total fat and fatty acids from the contents of the stomach and the small intestine was 42 mg., while that from the large intestine, in combination with the twenty-four hour fecal collection, was 61 mg. After the administration of 400 mg. of sodium ricinoleate, the average values were 49 and 69 mg., respectively. These slight increases account for only about 4 per cent of the dose administered.

TABLE II. RATE OF ABSORPTION OF SODIUM RICINOLEATE FROM GASTROINTESTINAL TRACT (400 MG. OF SODIUM RICINOLEATE ADMINISTERED BY STOMACH TUBE TO FASTING RATS)

TIME AFTER ADMINISTRATION (HR.)	STOMACH (MG.)	AMOUNT OF LIPIDS EXTRACTED			
		SMALL INTESTINE		TOTAL	
		UPPER (MG.)	LOWER (MG.)	(MG.)	(PER CENT OF DOSE)
Control	3	4	4	11	
½	260	26	13	299	77
3	239	38	26	303	78
6	185	42	30	257	66
16	26	8	11	45	9
24	8	7	9	24	4

In another experiment determinations were made for amounts of sodium ricinoleate and charcoal remaining in the stomach, in the upper, and in the lower half of the small intestine after varying periods of time following the dose. The amounts extracted are shown in Table II. The amount of lipids found in the control animal was somewhat less than that in the preceding experiment. There was considerable delay in the passage of sodium ricinoleate and charcoal from the stomach into the intestines. The stomach appeared to be considerably bloated during the first six hours after sodium ricinoleate. The charcoal was present only in the stomach and the upper half of the small intestine of animals killed one-half and three hours after the dose was administered. Six hours after the dose it was present also in the lower half of the small intestine. After sixteen hours most of the charcoal had passed into the large intestine, and the stomach was flat and not bloated. No charcoal was present in any segment after twenty-four hours, and the stomach was completely empty.

In the control animals, one-half hour after a dose was administered, there was charcoal present in all three segments. After three hours none of the charcoal was found in the stomach, and most of it was present in the lower half of the small intestine, with only a trace in the upper half. Nearly all had passed into the large intestine after six hours.

DISCUSSION

The data obtained in this study are in accord with previous studies[11, 12] on ricinoleic acid and castor oil and indicate that sodium ricinoleate is absorbed from the gastrointestinal tract and is not excreted in any appreciable amount in feces. Little information has been found regarding its fate after passing the intestinal barrier. Small amounts may be stored as a glyceride in fat depots, as was shown by Stewart and Sinclair[12] after the administration of castor oil. The major portion undoubtedly is oxidized in a manner similar to that of closely related unsaturated fatty acids. It is not likely that any free sodium ricinoleate occurs in the systemic blood stream at any time. Kolmer[9] has shown that 1 c.c. of 1:800 sodium ricinoleate produces complete hemolysis of 1 c.c. of defibrinated rabbit blood within twenty minutes at 37° C. Sodium ricinoleate frequently is used as a sclerosing agent for the treatment of varicose veins, due to its powerful hemolytic activity; it forms a jellylike coagulated mass when injected intravenously.[14] Accordingly, a substance with such properties is not likely to occur in a significant concentration in circulating blood after oral administration. Histologic examinations of cross sections of various portions of the intestinal tract, in rats on diets containing 0.5 Gm. sodium ricinoleate per day for periods as long as two months, do not show any tissue damage of any kind. The lack of sclerosing action would seem to indicate that sodium ricinoleate is undoubtedly converted by the epithelial cells into a triglyceride, in a manner similar to that of any other closely related fatty acid, and is thus carried into the lacteals in the form of chyle.

These studies do not explain the beneficial clinical action of sodium ricinoleate administered orally during the treatment of certain intestinal disorders. One cannot overlook the likelihood that it acts locally in the intestinal tract as a stimulant, thus altering intestinal rhythm either directly or through the sympathetic nervous system. Alteration in intestinal flora is also a good possibility, since it is well known that the character of the flora is an important factor for the general health of an individual. Another possibility is its reaction with toxins or poisons which in themselves may act as stimulants for abnormal intestinal rhythm or which may be absorbed into the blood stream, giving rise to the symptoms accompanying such disorders. The concentration of urinary indican in some patients with certain intestinal disturbances is quite high[15]; this generally returns to normal immediately after treatment with sodium ricinoleate.

From preliminary studies on the rate of absorption of sodium ricinoleate from different segments of the gastrointestinal tract, it appears that there would be considerable time for it to carry on its function as a therapeutic agent, as the emptying time of the stomach and the rate of absorption from the intestinal tract are considerably prolonged. The rate of passage of an indicator (animal charcoal) along the intestinal tract is considerably delayed in the presence of sodium ricinoleate, but the mechanism of this action has not yet been determined.

SUMMARY

Sodium ricinoleate was administered to white rats either by stomach tube or by feeding approximately 0.5 Gm. per day in the diet for two months. There is no evidence that any ricinoleic acid is excreted in the feces of these animals.

UNIVERSITY OF MICHIGAN LIBRARIES

Absorption studies indicate that orally administered sodium ricinoleate is almost completely absorbed from the gastrointestinal tract of rats during a period of twenty-four hours.

The rate of passage of an indicator (animal charcoal) through the intestinal tract of rats is considerably retarded by the oral administration of sodium ricinoleate.

REFERENCES

1. Morris, R. S., and Dorst, S. E.: Sodium Ricinoleate in Allergic Disease of the Intestinal Tract, Am. J. M. Sc. **178**: 631, 1929.
2. Dorst, S. E., and Morris, R. S.: Bacterial Hypersensitivity of the Intestinal Tract, Am. J. M. Sc. **180**: 650, 1930.
3. Morris, R. S., and Dorst, S. E.: Use of Sodium Ricinoleate in Bacterial Hypersensitiveness of the Intestinal Tract; Clinical Results, Ann. Int. Med. **4**: 396, 1930.
4. Burger, G. N.: The Treatment of Irritable Colon With Sodium Ricinoleate, J. Lab. & Clin. Med. **19**: 234, 1933.
5. Larson, W. P., and Eder, H.: Immunization Against Diphtheria With Toxin Detoxified With Sodium Ricinoleate, J. A. M. A. **86**: 998, 1926.
6. Larson, W. P., Evans, R. D., and Nelson, E.: The Effect of Sodium Ricinoleate Upon Bacterial Toxins, and the Value of Soap-Toxin Mixtures as Antigens, Proc. Soc. Exper. Biol. & Med. **22**: 194, 1925.
7. Sommer, E. W., and Sommer, H.: Influence of Nitrogenous and Lipid Compounds on the Potency of Botulinus Toxin, J. Infect. Dis. **43**: 496, 1908.
8. Netter, A., André, E., Césari, and Cotoni: Action of Sodium Ricinoleate on Bacteria and Toxins, Compt. rend. Soc. de biol. **96**: 184, 1927.
9. Kolmer, J. A.: Chemotherapeutic Studies With Sodium Ricinoleate (Soricin), J. Lab. & Clin. Med. **19**: 972, 1934.
10. Meyers, J. R., MacQuiddy, E. L., and Baker, C. P.: Sodium Ricinoleate, J. Lab. & Clin. Med. **19**: 462, 1934.
11. Paul, H., and McCay, C. M.: The Utilization of Fats by Herbivora, Arch. Biochem. **1**: 247, 1942.
12. Stewart, W. C., and Sinclair, R. G.: The Absence of Ricinoleic Acid From Phospholipids of Rats Fed Castor Oil, Arch. Biochem. **8**: 7, 1945.
13. Roberts, W. L., and Schuette, H. A.: Determination of Hydroxyl Number of Oils, Fats and Waxes, Ind. Eng. Chem., Anal. Ed. **4**: 257, 1932.
14. Froehlich, H. W., and Henrikson, H. W.: Sodium Ricinoleate as a Sclerosing Agent, Minnesota Med. **18**: 594, 1935.
15. Unpublished data.

OBSERVATIONS ON THE EFFECT OF LOWERED OXYGEN TENSION ON SICKLEMIA AND SICKLE CELL ANEMIA AMONG MILITARY FLYING PERSONNEL

MAJOR A. B. HENDERSON AND MAJOR H. E. THORNELL, MEDICAL CORPS,
ARMY OF THE UNITED STATES
DETROIT, MICH.

SURVEYS of large groups of Negroes in the United States reveal that the incidence of erythrocyte sickling varies from 4.3 to 14 per cent, with an accepted average between 7 and 9 per cent.[1, 2] Approximately one of fifteen develops the anemic state. Sickle cell anemia, therefore, is considered relatively uncommon when compared with the sickling trait or sicklemia. Reports on the incidence of the sickling phenomenon in Negroes outside the United States are few; to date, we have found only three such reports[3-5] in medical literature.

Because it has been demonstrated that diminishing the oxygen tension of wet preparations of the blood of patients with sickle cell anemia or sicklemia will cause the majority of erythrocytes to assume the sickle shape, it appeared important to determine whether the high altitudes reached by Negro flying personnel might increase the degree of sickling in vivo and lead to the development of symptoms. No reports on the effects of diminished oxygen tension in vivo on the sickling phenomenon could be found in the literature.

At the Tuskegee Army Airfield the flying personnel is predominantly Negro. The aviation cadets are all Negroes, and since 1941 pilots comprising fighter and bomber groups have received their preflight, primary, basic, and advanced training at that airfield and near-by Moton Field. On acceptance for aviation training all preaviation cadets undergo thorough physical and psychiatric examinations similar to those given at other fields. These men are a select group, and the percentage of eliminees in both the preflight and flight groups compares favorably with others in the Southeastern Training Command. Searching physical examinations are repeated at regular intervals during the training period; they include chest roentgenograms, serologic tests for syphilis, and urinalyses.

When the Negro was first assigned to aviation training, some doubt was expressed by physicians as to the physical capacity of those men with the sickling trait to tolerate high altitudes. No emphasis was placed on this problem, however, because it was expected that the routine use of oxygen would counteract any undesirable effects resulting from diminished oxygen tension. From the beginning, routine wet preparations of the blood were not obtained, and no incidence of the sickling phenomenon was determined. However, in over 1,500 cadets under training, none have been eliminated because of proved flying deficiencies arising from the sickling trait or active sickle cell anemia.

Early in 1945 we decided to study the incidence of sickling in Negro aviation cadets to determine whether there was any significant difference in the per-

Received for publication, Dec. 28, 1945.

centage of elimination between the groups having the sickling trait and the "normals." A small group of combat returnees was also observed for the sickling trait. It was our opinion that, if the relative incidence of sickling was preserved in various stages of training and in the returnees, this would aid in eliminating the sickling problem as a barrier to high altitude flying. In addition, to investigate further the effects of high altitude on persons with sicklemia, a small group of cadets was selected, on a volunteer basis, for observation while on a simulated flight in the low pressure chamber without oxygen.

Statistical Evidence That the Presence of Sicklemia in Flying Personnel Does Not Lead to Their Elimination From the Air Services.—Two hundred sixty cadets in various stages of training were examined for sickling by the wet preparation method. Two preparations were made from capillary blood on each subject. If after forty-eight hours these preparations failed to reveal sickling, they were labeled as negative. Slides were read at the end of twenty-four and forty-eight hours. It has been our experience, as well as that of Sherman,[6] that too many factors enter into the sickling phenomenon to attempt to gauge correctly the degree or time of appearance of sickling on the wet preparation; therefore, no attempt was made to determine accurately the percentage of sickling on any wet preparations. They were either declared negative or positive. Later, erythrocyte counts, thin blood films, and sedimentation rates were studied in a representative number of these men for evidence of active sickle cell anemia.

TABLE I. INCIDENCE OF SICKLEMIA IN CADETS AND COMBAT RETURNEES; ALL CADETS OBSERVED FOR MINIMAL PERIOD COVERING ONE PHASE OF TRAINING

NUMBER EXAMINED	STAGE OF TRAINING	NUMBER POSITIVE FOR SICKLING	PER CENT SICKLING	NUMBER NEGATIVE ELIMINEES	PER CENT NEGATIVE ELIMINEES	NUMBER SICKLING ELIMINEES	PER CENT SICKLING ELIMINEES
152	Primary	11	7.24	43	30.5	2	18.2
80	Basic	6	7.5	8	10.6	0	0.00
28	Advanced	2	7.14	0	00.0	0	0.00
52	Returnees	4	7.7	—	—	—	—
312		23	7.37	51	23.7	2	11.76

All cadets in our series were observed for a minimal period of three months. Of the 260 cadets examined, 152 were beginning their primary training. Another twenty-eight men were examined at the time of the last physical examination prior to receiving their commissions; the other 80 were in basic training. In addition to the 260 cadets examined, an additional fifty-two combat returnees were examined by the wet preparation method.

The results of our survey are presented in Table I. Note that a comparable incidence of sickling is preserved throughout all groups studied, although the total number in the individual groups is small. The percentage of eliminees with sicklemia in the primary and basic groups is less than in those not showing the sickling trait. This may not be significant but does indicate that those with sicklemia were at least maintaining a representative number in the various stages of training.

During the primary phase of training no high altitude missions were accomplished. In advanced training, however, numerous high altitude missions were flown; the percentage of sickling was comparable with the total number of cases of sickling found and to the general incidence of this condition. The four combat returnees with the sickle trait had flown both high and low altitude missions with the use of oxygen as is required of all pilots. The erythrocyte count, hemoglobin, reticulocyte count, and urine urobilinogen elimination were within normal level. The thin blood film revealed no typical sickled cells. There had been no unusual effects experienced while on high altitude missions. Total missions of these men ranged from 57 to 110 per person.

None of the twenty-three men positive for sickling had the drepanocytanemic or eunuchoid habitus described by others[7, 8]; they were classified as physically fit. There was no history of anemia, unusual or prolonged abdominal pains, joint pains, resistant leg ulcers, or jaundice. Erythrocyte counts performed on fifteen of these men varied between 4,200,000 and 5,840,000. Hemoglobin content and white blood counts were within the normal range.

Effect of Lowered Oxygen Tension Induced in the Low Pressure Chamber on the Blood of Persons With Sickle Cell Anemia and the Sickle Cell Trait.—Four subjects with sicklemia (Cases 1 to 4) and four controls (Cases 5 to 8) were utilized in this study. Four of the patients (Cases 1, 2, 5, and 6) had one previous experience in the low pressure chamber. They ranged in age between 20 and 24 years. One patient with active sickle cell anemia, a civilian resident of the near-by township, also volunteered for a simulated flight in the chamber. This subject had recently been discharged from the army because of anemia. He had a history of numerous acute episodes of joint pain, jaundice, and dyspnea on effort. At the time of examination he complained only of mild joint pains. He was able to perform his duties as an attendant at a near-by hospital without dyspnea. Physical examination revealed a canary-yellow color of the sclerae, apical systolic murmur, healed ulcers of the leg, and a drepanocytanemic habitus. The diagnostic parameter was 30.[9] Previous erythrocyte counts in our laboratory varied between 2.7 and 3.2 million with reticulocyte counts of 6.2 and 7.5 per cent. There was 8 per cent sickling of erythrocytes on the Wright's stained film; from 20 to 25 nucleated red blood cells were found per 1,000 erythrocytes. The packed cell volume was from 30 to 32 mm. The white count varied between 12,000 and 14,000 with 6 per cent eosinophiles.

Physical examination and hematologic studies of the subjects with sicklemia and controls were within the range of normal.

It was felt that total time of the simulated flight and the height reached would be dependent upon the physical capacities of the tested personnel to withstand the lowered oxygen tension. It was believed that it would be necessary to shorten the test when observations were made on the subject with active sickle cell anemia. Theoretically he was in a state of chronic oxygen want and the total oxygen content was lowered. Likewise, there was a probability that increased sickling might result in intravascular hemolysis and thrombosis. Before subjects entered the chamber, the following studies were made: erythrocyte count, hemoglobin determination, reticulocyte count, thin blood films stained

with Wright's stain, wet preparations for sickling, urinalyses for urobilinogen, icteric index, pulse rate, blood pressure, and activity of reflexes. Three subjects (Cases 2, 4, and 9) had electrocardiographic studies immediately before flight; in addition, two (Cases 4 and 9) had roentgen-ray examinations of the heart. All examinations listed were repeated after the descent. During the simulated flight reticulocyte preparations, dry thin blood films, and wet preparations were obtained at various levels. Thin blood films were reported positive for sickling, if the erythrocyte assumed the typical shape of the sickled cell. Close observation of the subjects for mental changes, visual disturbances, blood pressure changes, pulse rate, and reflex activity were also made. Oxygen was administered at the request of the individual subject or when, in the opinion of the examiners, it was thought necessary.

Oxygen arterial saturation levels were obtained on Cases 4 and 9 by means of the oxygen tensiometer which is a relatively inexact method for determination of the percentage of arterial oxygen saturation.

Forty-eight hours after completion of the test, erythrocyte counts, urine urobilinogen levels, and icteric indices were repeated on the subjects with sicklemia and active sickle cell anemia. It is recognized that the icteric index and the amount of urobilinogen excretion in the urine do not provide accurate methods for estimating the degree of hemolysis, but facilities were not available for fecal urobilinogen determinations or plasma hemoglobin measurement.

RESULTS

The results of our studies are presented in Table II. All subjects except three (Cases 4, 5, and 9) were administered oxygen at 15,000 feet. Two subjects (Cases 4 and 9) were taken to 16,000 feet where after five minutes it was considered necessary to administer oxygen to one (Case 4). The other (Case 9), the subject with active sickle cell anemia, contrary to expectation, revealed less clinical evidence of oxygen want than any of the subjects. His ability to read the type I Jaeger chart at 15,000 feet surpassed that of the others. He was more relaxed and appeared fairly comfortable at the higher levels. Another subject (Case 5), an excitable and apprehensive person, needed oxygen at 10,000 feet. Two individuals (Cases 3 and 6) developed pronounced extrasystoles at the 15,000 foot level; these extrasystoles disappeared on descent. One subject (Case 9) was given oxygen in amounts comparable to that at ground level for a period of ten minutes after five minutes without oxygen at 16,000 feet.

In Table II, the results reveal no evidence of increased blood destruction. The erythrocyte count was frequently but not consistently increased after descent, but the reticulocyte count showed no change. Signs and symptoms of oxygen deficiency were not unusual for the subjects with sicklemia and tended to follow the general pattern of our controls and other healthy subjects in the Army under diminished oxygen tension. A gradual increase in pulse rate, blood pressure, and activity of reflexes usually occurred and at 15,000 feet vision was sufficiently blurred to cause difficulty in reading the type I Jaeger chart.

TABLE II. RESULTS OBTAINED BEFORE, DURING, AND AFTER SIMULATED FLIGHT IN LOW PRESSURE CHAMBER

		BEFORE SIMULATED FLIGHT								5000 FT. 15 MIN.			10,000 FT., 50 MIN. (BAR. PRESS. 522.6 MM. Hg)			15,000 FT., 30 MIN.			16,000 FT., 5 MIN. (BAR. PRESS. 411.8 MM. Hg)			16,000 FT. UNDER O_2, 10 MIN.		AFTER SIMULATED FLIGHT						48 HOURS AFTER FLIGHT		
CASE		ERYTHROCYTES (PER C.MM.)	HEMOGLOBIN (PER CENT)	SICKLING (PER CENT)	RETICULOCYTES (PER CENT)	ICTERIC INDEX	UROBILINOGEN	ART. OXYGEN SATURATION (PER CENT)		SICKLING (PER CENT)	ART. OXYGEN SATURATION (PER CENT)	RETICULOCYTES (PER CENT)	SICKLING (PER CENT)	ART. OXYGEN SATURATION (PER CENT)	RETICULOCYTES (PER CENT)	SICKLING (PER CENT)	ART. OXYGEN SATURATION (PER CENT)	RETICULOCYTES (PER CENT)	SICKLING (PER CENT)	ART. OXYGEN SATURATION (PER CENT)	RETICULOCYTES (PER CENT)	SICKLING (PER CENT)	ART. OXYGEN SATURATION (PER CENT)	ERYTHROCYTES (PER C.MM.)	HEMOGLOBIN (PER CENT)	RETICULOCYTES (PER CENT)	SICKLING (PER CENT)	ICTERIC INDEX	UROBILINOGEN	ERYTHROCYTES (PER C.MM.)	UROBILINOGEN	SICKLING (PER CENT)
With sicklemias	1	5.2 Mil.	100	N	0.2	2	N	--	N	-	0.2	N	--	0.3	N	--	0.2	--	--	--	--	--	--	--	5.4 Mil.	110	0.1	N	3	N	5.0 Mil.	N	N
	2	4.6	80	N	0.1	5	N	--	N	-	0.2	N	--	0.1	N	--	0.3	--	--	--	--	--	--	--	4.8	90	0.3	N	3	N	4.6	N	N
	3	4.4	85	N	0.4	8	N	--	N	-	0.3	N	--	0.5	N	--	0.5	--	--	--	--	--	--	--	4.1	85	0.2	N	6	N	4.3	N	N
	4	4.8	95	N	0.6	4	N	94	N	93	0.9	N	86	0.4	N	72	0.6	--	--	--	--	--	--	--	5.0	95	0.4	N	4	N	4.7	N	N
Controls	5	5.1 Mil.	90	N	0.4	6	N	--	N	-	0.2	N	--	0.2	--	--	--	--	--	--	--	--	--	--	4.8 Mil.	90	0.2	N	6	N	--	--	--
	6	5.4	110	N	0.1	7	N	--	N	-	0.1	N	--	0.1	N	--	0.1	--	--	--	--	--	--	--	5.7	110	0.2	N	6	N	--	--	--
	7	4.3	90	N	0.2	4	N	--	N	-	0.1	N	--	0.2	N	--	0.4	--	--	--	--	--	--	--	4.8	100	0.3	N	6	N	--	--	--
	8	4.9	90	N	0.6	8	N	--	N	-	0.9	N	--	0.8	N	--	0.8	--	--	--	--	--	--	--	5.2	100	0.8	N	8	N	--	--	--
With active sickle anemia	9	3.2	50	8	6.4	20	Pos. 1/40	96	8	96	7	9.8	85	7	12	74	6	15	74	7.2	7.2	84	3.9	60	6.0	9	21	Pos. 1/40	3.6	Pos. 1/40	8

Total time of simulated flight, two hours, eleven minutes.
Per cent sickling read on thin blood smear.
N, Not significant.

UNIVERSITY OF MICHIGAN LIBRARIES

There was a moderate increase in the percentage of sickling as observed by the Wright's stained thin film taken at various levels and degrees of lowering of the oxygen saturation in Case 9. After ten minutes of oxygen at 16,000 feet a decrease in the degree of sickling was noted. These findings are demonstrated in Table II and Fig. 1. During the descent no oxygen was administered to this subject. His increase in erythrocytes was greater than the others and follow-up studies showed no evidence of any marked increase of hemolysis. Two months later his erythrocyte count was 3.6 million, and 8 per cent sickling on

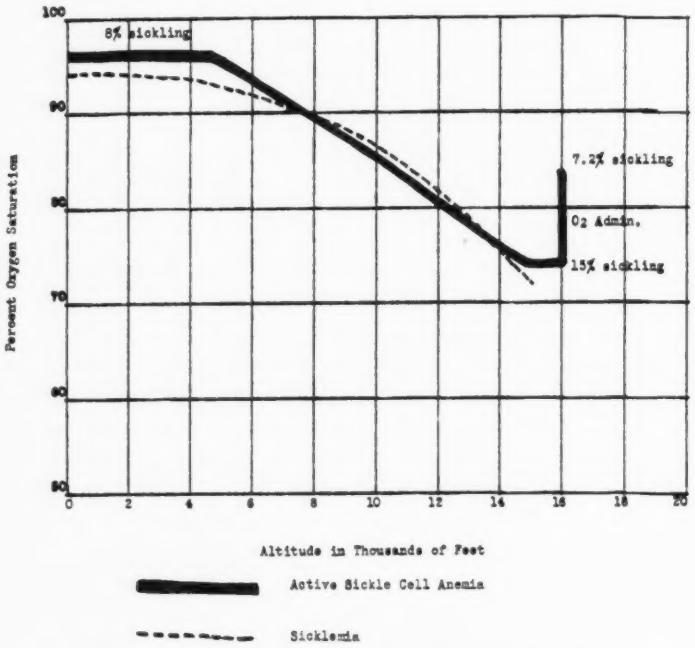

Fig. 1.—Percentage of oxygen saturation of blood at various altitudes up to 15,000 feet in a case of sicklemia and 16,000 feet in a subject with active sickle cell anemia as determined by the oxygen tensiometer. Active sickle cell anemia subject given oxygen (approximately 40 per cent) ten minutes at 16,000 feet. The sicklemia revealed no immediate sickling.

the Wright's stain. Examination of the wet and dry preparations taken immediately on the other subjects revealed no change. After twenty-four hours wet preparations obtained from the subjects with sicklemia were positive for sickling.

Electrocardiographic and roentgenologic studies made before the test revealed no significant change after completion of the test.

COMMENT

From our observations the incidence of sicklemia in the various training groups of Negroes at the Tuskegee Army Airfield is consistent with the reported incidence of this abnormality. Although the number of cases studied was small, it appears that the sickling trait played no significant part in the elimination of Negro cadets for flying deficiencies.

Wintrobe[10] states that there is no evidence of definite geographic or racial differences at sea level in regard to the amount of hemoglobin or the number of erythrocytes in the circulating blood. Hurtado and associates[11] have presented an excellent study on the influence of anoxemia on the hemopoietic system. They believe that, in states of temporary anoxemia similar to our studies, any polycythemia noted is probably the result of a release of stored red blood cells and hemoconcentration; that corresponding to a repeated or constant exposure to a low pressure environment is related to an erythropoietic hyperactivity. In temporary anoxemia there are individual responses, and even a decrease in erythrocytes may occur.

Our subjects who volunteered for a simulated flight in the low pressure chamber were all healthy physically fit young males, with the exception of the man with active sickle cell anemia who could be classified as being in a state of chronic oxygen want. Our observations were primarily directed toward the detection of any evidence of massive erythrocyte destruction as a result of a lowering of the arterial oxygen tension to the levels of human intolerance in persons with the sickling trait. No sickling was observed immediately in any of the subjects with sicklemia and increased erythrocyte destruction was not detected by our methods of testing. The subjects with sicklemia varied in their response to high altitude as did the controls. Physical responses to lowered oxygen tension were not remarkable.

The results observed in the subject with active sickle cell anemia deserve further study and indicate that there is a gradual increase in sickling proportional to the decrease in the oxygen tension. However, an adequately sensitive compensatory mechanism resulted in an increase in erythrocytes which sufficed apparently for his oxygen needs. This increase was evidently the result of a release of stored red blood cells. It would appear that, although this subject was already in a state of oxygen want, he was able to respond even better to a diminished oxygen tension than did the controls or subjects with sicklemia. There was no evidence of massive erythrocyte destruction in this subject, although, as previously stated, it is recognized that determination of urobilinogen excretion in the urine and the icteric index do not provide accurate methods for estimating rates of red blood cell hemolysis. However, an erythrocyte and reticulocyte count obtained simultaneously with these two tests should reveal the presence of massive hemolysis fairly accurately.[12]

It is unfortunate that more acurate methods could not be used in this study. The precautions outlined by Sherman[6] in obtaining blood from the patient with sickle cell anemia would have presented much better evidence of increased intravascular sickling, since the role of atmospheric pressure in causing this phenomenon cannot be excluded.

However, the decrease in sickling noted after administration of oxygen minimizes to some extent the effect of atmospheric conditions on the erythrocytes in vitro and is compatible with the studies of Reinhard and associates[13] who administered high oxygen concentrations to subjects with sickle cell anemia and obtained a decrease in the degree of sickling.

SUMMARY AND CONCLUSIONS

In a study of the incidence of sicklemia among Negro cadets in various stages of training and combat returnees, we found an incidence of 7.37 per cent which compares favorably with the over-all percentage of sickling in the Negro race. There was no evidence of an increased elimination of cadets because of the sickling trait.

Under lowered arterial oxygen tension near the levels of human intolerance, none of the subjects with sicklemia who were tested revealed evidences of immediate intravascular sickling or increased erythrocyte hemolysis.

A volunteer subject with active sickle cell anemia withstood the lowered oxygen tension even better than did the individuals with sicklemia or the control subjects, although there was suggestive evidence of increased intravascular sickling. Administration of oxygen for a short period resulted in more normal erythrocytes. This suggests that adequate administration of oxygen will protect patients who have active sickle cell anemia under conditions which ordinarily will result in a lowered oxygen tension and increased intravascular sickling. Even without oxygen therapy there was no evidence of massive erythrocyte destruction.

REFERENCES

1. Kracke, Roy R.: Diseases of the Blood, ed. 2, Philadelphia, 1941, J. B. Lippincott Company, pp. 325-333.
2. Cooley, T. B., and Lee, P.: The Sickle Cell Phenomenon, Am. J. Dis. Child. **32**: 334-340, 1926.
3. McGavack, T. H., and German, W. M.: Sicklemia in the Black Carib Indian, Am. J. M. Sc. **208**: 350-355, 1944.
4. Evans, R. W.: The Sickling Phenomenon in the Blood of West African Natives, Tr. Roy. Soc. Trop. Med. & Hyg. **37**: 281-286, 1944.
5. Tomlinson, W. J.: The Incidence of Sicklemia and Sickle Cell Anemia in 3000 Canal Zone Examinations Upon Natives of Central America, Am. J. M. Sc. **209**: 181-186, 1945.
6. Sherman, I. J.: The Sickling Phenomenon, With Special Reference to the Differentiation of Sickle Cell Anemia From the Sickle Cell Trait, Bull. Johns Hopkins Hosp. **67**: 309-324, 1940.
7. Winsor, T., and Burch, G. E.: The Habitus of Patients With Sickle Cell Anemia, Human Biol. **16**: 99-114, 1944.
8. Sharp, E. A., and Vonder Heide, E. C.: Eunuchoid Habitus Associated With Sickle-Cell Anemia and the Sickling Trait, J. Clin. Endocrinol. **4**: 505-510, 1944.
9. Winsor, T., and Burch, G. E.: Diagnostic Physiocochemical Blood Tests in Sickle Cell Anemia, Am. J. M. Sc. **207**: 152-160, 1944.
10. Wintrobe, M. M.: Blood of Normal Men and Women; Erythrocyte Counts, Hemoglobin and Volume of Packed Red Cells of 229 Individuals, Bull. Johns Hopkins Hosp. **58**: 118-130, 1933.
11. Hurtado, A., Merino, C., and Delgado, E.: Influence of Anoxemia and Hemopoietic Activity, Arch. Int. Med. **75**: 284-323, 1945.
12. Watson, Cecil J.: Personal communication.
13. Reinhard, E. H., Moore, C. V., Dubach, R., and Wade, L. J.: The Effect of Breathing 80 to 100 per cent Oxygen on the Erythrocyte Equilibrium in Patients With Sickle Cell Anemia, Proc. Central Soc. Clin. Research **15**: 13-14, 1942.

THE BONE MARROW IN HEMOPHILIA DURING LIFE*

A Study of the Cellular Elements and the Coagulation Time of the Marrow Blood

Louis R. Limarzi, M.D., Henry G. Poncher, M.D., and Carroll L. Birch, M.D.
Chicago, Ill.

THE bone marrow in hemophilia during life has not been studied. In fact, hemophilia is the only condition listed in which sternal puncture is contraindicated.[2] Bone marrow studies in this disorder have been limited to autopsy material. Custer and Krumbhaar[1] reviewed the literature and studied in detail the histopathology of the hemopoietic tissues in three patients with hemophilia who died in various phases of the hemorrhagic disorder. As hemophilia is characterized by a prolongation of the coagulation time of blood, the megakaryocytes of the bone marrow were of particular interest. The coagulation time of the marrow fluid was determined and compared with the coagulation time of the peripheral blood.

METHODS

The method of sternal aspiration and preparation of the marrow specimens has been reported in detail in a previous paper.[3] Under aseptic technique and procaine anesthesia the sternum is punctured in the second or third interspace with a specially devised 16 gauge needle. The needle is forced into the bone perpendicular to the sternum until a sudden "give" indicates that the marrow cavity has been reached. The stylet is then removed. With a tightly fitting, dry 5 c.c. syringe, about 1 c.c. of material is aspirated and immediately placed in a paraffin-lined tube containing a minute amount of heparin. Without delay a second dry syringe (10 c.c. size) is attached to the sternal needle, and from 3 to 5 c.c. of marrow fluid are aspirated. This "marrow blood" is practically devoid of hemopoietic elements, since most of the marrow elements are removed in the first 1. c.c.; it is expelled carefully into a clean dry sterile serologic tube 21 mm. in diameter, care being taken to prevent the formation of air bubbles. The tube is tilted every one or two minutes, and when the tube can be inverted without the blood flowing out, coagulation is considered complete.

The 1 c.c. of heparinized material consisting of sinusoidal blood, hemopoietic marrow, and fat is pipetted into a hematocrit tube and centrifuged from 2,000 to 2,500 r.p.m. for five minutes. Centrifugation separates the following layers reading from top down in the tube: (1) fat, (2) plasma, (3) myeloid-erythroid cells (this layer also contains the megakaryocytes), and (4) erythrocytes (sinusoidal blood). The heights of the several layers are recorded. The fat and plasma are removed separately and discarded. The myeloid-erythroid layer, together with a small amount of the erythrocyte layer, is pipetted off separately, transferred to a paraffined watch glass, mixed, and from this material films are made and stained with Wright's stain and studied microscopically for cell distribution and types.

From the Department of Internal Medicine and the Department of Pediatrics, University of Illinois, College of Medicine.

These studies were made possible, in part, through a grant from Armour and Company, Chicago, Ill.

Received for publication, March 30, 1946.

*Paper read by title before the Central Society for Clinical Research in Chicago, Ill., Nov. 3, 1944.

Immediately preceding the marrow aspiration, 10 c.c. of peripheral blood are withdrawn by venipuncture into a dry, sterile syringe. Five cubic centimeters of the blood are expelled carefully into a clean, dry, sterile serologic tube 21 mm. in diameter, and the coagulation time is determined (Howell's method). The remaining 5 c.c. of blood are transferred to a bottle containing a measured amount of ammonium and potassium oxalate mixture as an anticoagulant. This blood is shaken thoroughly and used immediately for the following studies: hemoglobin determination, erythrocyte and leucocyte counts, differential counts, and reticulocyte and platelet estimations. The volume of packed cells, sedimentation rate, and icterus index are determined by Wintrobe's method.[11] Mean corpuscular volume, hemoglobin, and hemoglobin concentration are then computed.

When the stained films of the marrow are studied, the ratio of the myeloid to the erythroid cells, the dispersion of the nucleated red cells, the dispersion of the myeloid series, and a differential count of the megakaryocytes designated as young, adult, and degenerated are recorded. Under young forms are included the promegakaryocytes, the earliest type of megakaryocyte, seen chiefly in essential thrombocytic purpura and the intermediate forms seen usually in chronic hemorrhage.

In recording the erythroid series the terms pronormoblast, basophilic, polychromatophilic, and orthochromatic (acidophilic) normoblasts are used in order of their increasing maturity. Similarly for the myeloid series the terms myeloblast, promyelocyte, myelocyte, metamyelocyte, band, and polymorphonuclear are used. Plasma cells, tissue cells, lymphocytes, and monocytes are noted when present but are not included in the counts used to determine the foregoing percentage relationships.

Other laboratory determinations such as clot retraction and bleeding time, tourniquet test, plasma ascorbic acid, and prothrombin time were performed.

RESULTS

Four patients with hemophilia were studied during various stages of activity. All four subjects had typical hereditary and individual histories of bleeding episodes and had been followed for a number of years in the pediatric, orthopedic, and medical wards.[2] The two younger boys had hemophilic joints; the Negro with hemophilia had a leg operation at which time the hemophilic condition was discovered; and the fourth patient, a 51-year-old man, had a carcinoma of the penis. In Table I the coagulation times of the peripheral blood and bone marrow blood are compared with similar studies in a variety of hematologic, disorders and other clinical conditions. In two patients with hemophilia studied during the remission phase, the blood studies and the coagulation time (Howell's method) of the peripheral blood were 20 minutes and 25 minutes, respectively, while the coagulation of the bone marrow blood was 25 minutes and 29 minutes, respectively. These values are normal and within the range of technical error. In the two subjects with hemophilia studied during an exacerbation of the hemorrhagic disorder, with a macrocytic anemia in one subject and a normocytic anemia in the other, the coagulation time of the blood was 90 minutes and 150 minutes, respectively, while the marrow blood coagulation time was 150 minutes in both subjects.

In normal controls, patients with polycythemia vera, normocytic, microcytic hypochromic (due to hemorrhage), macrocytic anemias, leucemias, and thrombocytopenic purpura, the coagulation time of the marrow blood varied between 15 seconds and 3 minutes while that of the peripheral blood taken

TABLE I. LABORATORY FINDINGS IN BLOOD AND BONE MARROW IN PATIENTS WITH HEMOPHILIA AND VARIOUS CLINICAL DISORDERS

DISEASE	BLOOD							BONE MARROW	
	AGE AND RACE	BLEEDING TIME—DUKE METHOD (MINUTES)	COAGULATION TIME—HOWELL METHOD (MINUTES)	CLOT RETRACTION TIME (HOURS)	CEVITAMIC ACID	PROTHROMBIN TIME	TOURNIQUET TEST	COAGULATION TIME—HOWELL METHOD (MINUTES)	CLOT RETRACTION (HOURS)
Hemophilia T.S.	51 W.	3.0	20	6	Normal	Normal	Neg.	25	1½
Hemophilia I.H.	20 C.	3.5	25	2½	Normal	Normal	Neg.	29	3
Hemophilia H.B.	12 W.	4.0	190	1	Normal	Normal	Neg.	150	6
Hemophilia T.B.	3 W.	5.5	150	18	Normal	Normal	Neg.	150	4
Normals (4)*			16					2.5	
Pernicious anemia (2)			15					2.15	
Normocytic anemia (1)			15					2	
Hemorrhagic anemia (2)			14					1.30	
Refractory anemia (1)			5					3	
Leucemia (1)			12					2	
Thrombocytopenic purpura (1)			10					3	
Polycythemia vera (2)			7					2.15	
Cirrhosis of liver with jaundice (1)			9					3.30	
Infectious mononucleosis			6.30					13	
Pulmonary tuberculosis (1)			4.25					3	

*Number of patients.

from the arm vein ranged from 5 to 18 minutes. In a subject with pulmonary tuberculosis the relationship of the coagulation time of the marrow blood to that of the peripheral blood was from 3 to 4 minutes, 25 seconds, and in a subject with infectious mononucleosis the marrow blood coagulated in 13 minutes while the peripheral blood clotted in 6 minutes, 30 seconds.

The peripheral blood (Table II) in the two patients with hemophilia revealed a macrocytic and hypochromic anemia in one patient and a normocytic anemia in another; in neither patient was the anemia severe. In both of these patients there was a slight polymorphonuclear leucocytosis and a normal to increased number of blood platelets. The other blood studies showed no significant change from normal.

The bone marrow (Table II) in two subjects with hemophilia, studied during the remission phase with normal blood findings and coagulation time, revealed a normal quantitative and qualitative erythroid and myeloid pattern. The megakaryocytes were quantitatively and morphologically normal. There

TABLE II. BLOOD AND BONE MARROW FINDINGS IN PATIENTS WITH HEMOPHILIA AND IN OTHER CLINICAL CONDITIONS

	PERIPHERAL BLOOD																		
														PERCENTAGE					
	PATIENT, AGE, AND RACE	HEMOGLOBIN (GM.)	ERYTHROCYTES (MILLIONS)	LEUCOCYTES (THOUSANDS)	PLATELETS (THOUSANDS)	HEMATOCRIT (%)	MEAN CORPUSCULAR VOLUME (CUBIC MICRONS)	MEAN CORPUSCULAR HEMOGLOBIN (MICRO-MICROGRAMS)	MEAN CORPUSCULAR HB. CONCENTRATION (%)	SEDIMENTATION RATE (UNCORRECTED) MM./HR.	SEDIMENTATION RATE (CORRECTED) MM./HR.	RETICULOCYTES (%)	ICTERUS INDEX (UNITS)	STAB FORMS	POLYMORPHONUCLEAR NEUTROPHILES	EOSINOPHILES	BASOPHILES	MONOCYTES	LYMPHOCYTES
Patients with hemophilia	T. S., 51, W.	13.5	4.87	8.0	Normal	46.0	94.0	27.0	28.0	28.0	26.0	0.5	5.0	3.0	56.0	11.0	0.0	3.0	27.0
	T. H., 20, C.	14.0	4.74	6.6	Normal	41.0	86.0	29.0	34.0	16.0	8.0	1.0	5.0	4.0	62.0	0.0	0.0	4.0	30.0
	H. B., 12, W.	12.0	4.10	10.5	Normal	41.0	100.0	29.0	29.0	14.0	8.0	1.5	7.5	0.0	72.0	2.0	1.0	2.0	23.0
	T. B., 13, W.	11.0	3.27	12.6	Increased	30.0	91.0	33.0	36.0	32.0	8.0	2.0	7.5	7.0	65.0	0.0	0.0	4.0	25.0
Normals (10)*		14.91	5.0	7.45	Normal	44.6	88.5		33.5		7.3	0.3	6.5	5.1	54.5	1.4	0.6	8.9	29.5
Essential thrombopenic purpura (5)																			
Chronic thrombopenic purpura (1)																			
Secondary thrombopenic purpura (1)																			
Symptomatic thrombopenic purpura (3)																			
Chronic hemorrhage (5)																			
Acute hemorrhage (3)																			

TABLE II. BLOOD AND BONE MARROW FINDINGS IN PATIENTS WITH HEMOPHILIA AND IN OTHER CLINICAL CONDITIONS—CONT'D

		BONE MARROW																		
				ERYTHROID (PER CENT)				MYELOID (PER CENT)										MEGAKARYOCYTES (PER CENT)		
	PATIENT, AGE, AND RACE	MYELOID ERYTHROID (VOL. %)	FAT VOLUME (%)	PRONORMOBLASTS	BASOPHILIC NORMOBLASTS	POLYCHROMATIC NORMOBLASTS	ORTHOCHROMATIC NORMOBLASTS	MYELOBLASTS	PROMYELOCYTES	NEUTROPHILIC MYELOCYTES	EOSINOPHILIC MYELOCYTES	NEUTROPHILIC METAMYELOCYTES	EOSINOPHILIC METAMYELOCYTES	POLYMORPHONUCLEAR NEUTROPHILES	EOSINOPHILES	BASOPHILES	MYELOID-ERYTHROID RATIO (%)	IMMATURE	MATURE	DEGENERATED AND FREE NUCLEI
Patients with hemophilia	T. S., 51, W.	8.0	2.0	3.0	12.0	85.0	0.0	0.0	2.0	12.0	7.0	56.0†	3.0	13.0	1.0	1.0	67/23	11.0	61.0	28.0
	T. H., 20, C.	6.0	2.0	1.0	8.0	91.0	0.0	0.0	2.0	30.0	1.0	49.0	1.0	15.0	2.0	0.0	63/37	5.0	75.0	20.0
	H. B., 12, W.	12.0	1.0	7.0	15.0	75.0	3.0	0.0	0.0	15.0	6.0	60.0	2.0	15.0	1.0	1.0	57/43	10.0	64.0	26.0
	T. B., 13, W.	14.0	2.0	9.0	25.0	58.0	8.0	0.0	1.0	11.0	1.0	78.0	3.0	10.0	1.0	1.0	46/54	7.0	57.0	36.0
Normals (10)*		6.8	3.2	2.6	13.2	79.3	5.9	0.4	0.9	7.8	3.0	56.8	5.0	23.1	2.5	0.5	2.75:1.0	24.0	48.0	28.0
Essential thrombopenic purpura (5)		18.0																44.4	33.8	21.8
Chronic thrombopenic purpura (1)		7.0																52.0	24.0	24.0
Secondary thrombopenic purpura (1)		30.0																34.0	33.0	33.0
Symptomatic thrombopenic purpura (3)		11.0																31.5	42.0	26.5
Chronic hemorrhage (5)		11.8																31.0	48.0	21.0
Acute hemorrhage (3)		12.0																11.0	75.0	14.0

*Number of patients.
†Includes band forms.

were large numbers of normal appearing platelets and many free megakaryocyte nuclei. The mature types of megakaryocytes were increased in number. The two patients with hemophilia who were studied during a bleeding episode revealed a hyperplastic bone marrow (myeloid-erythroid volume). The mature types of megakaryocytes were increased and, as in the first two subjects with hemophilia, there were large numbers of formed platelets and free megakaryocyte nuclei.

COMMENT

In the reported necropsies on hemophilia either the bone marrow was not examined or little of histologic significance concerning the marrow picture was reported.[1] Ricker[9] stated that the vertebral and femoral marrows were not hyperplastic. Custer and Krumbhaar,[1] who were the first to make a comprehensive histologic study of the bone marrow as well as of the other hemopoietic tissues, remarked that the histopathology of the hemopoietic tissues in hemophilia was an unexplored field. In the three patients reported on by Custer and Krumbhaar the blood forming tissues all showed normal regenerative ability, the first two were predominately erythroblastic in character and the third showed a leucoblastic type of regeneration. All three patients showed a marked increase of early types of the thrombocytic series (megakaryoblasts) and megakaryocytes. The morphology of the megakaryocytes did not vary from the normal, and Wright's figures (pseudopods of the megakaryocyte cytoplasm) were occasionally observed. Autopsies were performed on Custer and Krumbhaar's patients following hemorrhagic episodes.

In our four patients with hemophilia the bone marrow pattern (Table II) could be correlated with the stage of the hemorrhagic disease. During the bleeding phase of the disorder with a prolonged coagulation time, the bone marrow showed a uniform hyperplasia of all elements with varying degrees of myeloid and erythroid hyperplasia. The megakaryocytes were increased in number but were morphologically normal. During the remission phase with normal blood findings and a normal coagulation time, the bone marrow revealed a normal erythroid and myeloid pattern, and the megakaryocytes were normal in number and morphology. In all the marrows the number of formed and morphologically normal platelets were increased with numerous free megakaryocyte nuclei. The augumented megakaryocyte nuclei may be due in part to the mechanical damage or rupture of the large megakaryocytic mass with extrusion and breaking up of the large multilobulated nucleus. This mechanical mechanism does not account for the large numbers of formed platelets. The predominant number of adult megakaryocytes which exceed the total of both immature and degenerated types of megakaryocytes is evidence of an accelerated rate of platelet formation (Table II). Many of the formed platelets in the marrow probably degenerate in situ in view of the fact that the peripheral blood does not reflect the increased plateletogenesis in the bone marrow. In normal control subjects[4] and in patients with thrombocytopenic purpura[5] (Table II), the combined number of immature and degenerated types of megakaryocytes exceeds the mature or adult types of megakaryocytes. It is

interesting to note that in acute thrombocytopenic purpura with hemorrhage there is a megakaryocytic hyperplasia of the young forms (immature), and in the less acute phase or chronic stage the mature types appear; however, the platelets are markedly decreased or absent in both the bone marrow and peripheral blood. This is due to faulty maturation and a lack of platelet formation of the megakaryocytes in the bone marrow.[6]

The origin, formation, and morphologic description of the megakaryocytes in human adult bone marrow under normal and pathologic conditions has been discussed and illustrated elsewhere.[6] Mature megakaryocytes are thought to be formed from the myeloblast through a process of heteroplastic hemopoiesis in contrast to the homoplastic hemopoietic development of the myeloid[10] and erythroid cells.[7] If this concept is correct, any disturbance involving the stem cell (myeloblast) will eventually interfere with the normal development and platelet formation of the megakaryocyte in the bone marrow. The formation of platelets occurs by a process of budding or by detachment of portions of the cytoplasm (Wright's figure) of the megakaryocytes in the bone marrow. It occurs as a normal process and more frequently by cytolysis of the cytoplasm of these cells as observed in aspirated human bone marrow.[6]

The increased number of platelets and free megakaryocyte nuclei seen in the marrow of patients with hemophilia is similarly observed in the marrow following acute hemorrhage.

The coagulation time of the marrow blood in patients with hemophilia equaled or exceeded the coagulation time of the blood taken from the arm vein. In one subject the marrow coagulation time was one hour longer than that of the peripheral blood. On the other hand, in normal controls, blood disorders, and various clinical conditions the coagulation time of the peripheral blood was several times that of the marrow blood. The only exception was in a patient with infectious mononucleosis in which the marrow coagulation time exceeded that of the peripheral blood.

The cause of the prolonged coagulation time of the marrow blood in hemophilia and the accelerated rate of platelet formation in the bone marrow is not entirely clear. One may speculate that there exists some relationship which results in a type of platelet that is morphologically normal but physiologically abnormal (in other words, an immature type of platelet which is physiologically and abnormally resistant due to the accelerated rate of the plateletogenesis in the bone marrow), and this is reflected in the peripheral blood by increased stability of the platelets. The abnormal plateletogenesis in the marrow may be due to the abnormal environment (hemophilic marrow plasma) or may equally well be regarded as an abnormal environment (prolonged marrow coagulation) due to the abnormal plateletogenesis which results in the slow liberation of thromboplastin from the platelets.

CONCLUSIONS

1. In patients with hemophilia who have a coagulation time of 2 hours, 15 minutes, sternal aspiration is not a contraindication.

2. The megakaryocytes in the bone marrow in patients with hemophilia are morphologically normal.

3. The megakaryocytes in the bone marrow in subjects with hemophilia appear to have an accelerated rate of maturation and platelet formation when compared with the maturation rate and platelet formation seen in the bone marrow in various other clinical conditions.

4. The hyperplasia of the myeloid and erythroid elements can be definitely correlated with the hemorrhagic tendency of the disease.

5. The coagulation time of the marrow blood in patients with hemophilia exceeds the coagulation time of the peripheral blood in contrast to the reverse relationship observed in normal individuals and in various clinical conditions.

6. The abnormal rate of plateletogenesis in the bone marrow and the increased coagulation time of the marrow blood may be associated with the pathologic physiology of hemophilia.

REFERENCES

1. Custer, R. P., and Krumbhaar, E. B.: The Histopathology of the Hemopoietic Tissues in Hemophilia; an Unexplored Field, Am. J. M. Sc. **189**: 620, 1935.
2. Birch, C. L.: (a) Hemophilia, J. A. M. A. **99**: 1566, 1932.
Birch, C. L.: (b) Hemophilia, University of Illinois Bulletin, Vol. 34, No. 55, Illinois Medical and Dental Monographs, Vol. 1, No. 4, Urbana, Ill., 1937, University of Illinois Press.
3. Limarzi, L. R.: The Diagnostic Value of Sternal Marrow Aspiration, Illinois M. J. **75**: 38, 1939.
4. Limarzi, L. R., Jones, R. M., Paul, J. T., and Poncher, H. G.: Sternal Marrow in Banti's Syndrome and Other Splenomegalic States; the Effect of Splenectomy, Am. J. Clin. Path. **13**: 231, 1943.
5. Limarzi, L. R., and Schleicher, E. M.: The Reaction of the Peripheral Blood and Bone Marrow in Chronic Hemorrhage and in Essential Thrombopenic Purpura, J. A. M. A. **114**: 12, 1940.
6. Limarzi, L. R.: Thrombocytopenic Purpura, M. Clin. North America **28**: 153, 1944.
7. Limarzi, L. R., and Levinson, S. A.: An Undescribed Type of Erythropoiesis Observed in Human Sternal Marrow, Arch. Path. **36**: 127, 1943.
8. Revol, L.: L'exploration de Moelle osseuse par ponction sternale, Paris, 1938, J. B. Baillière et fils.
9. Ricker, G.: Ueber die hemorrhagische Infarzierung des Nierenlagers und andere Kapillare Diapedesis, Blutungen grossen Umfanges an und in Organen der Bauchhöhle, Beitr. z. path. Anat. u. z. allg. Path. **50**: 579, 1911.
10. Wolff, J. R., and Limarzi, L. R.: Anemia in Pregnancy; a Correlation of the Peripheral Blood and Bone Marrow Findings, J. A. M. A. **128**: 482, 1945.
11. Wintrobe, M. M.: Anemia, Classification and Treatment on the Basis of Differences in Average Volume and Hemoglobin Content of Red Corpuscles, Arch. Int. Med. **54**: 256, 1934.

PROPERTY OF THE
Kilmer Memorial Laboratory
of the Binghamton City Hospital
MUST NOT BE REMOVED
FROM LABORATORY

AN UNUSUAL CASE OF GENERALIZED ANASARCA APPEARING IN AN IMPOUNDED DOG WITH CIRRHOSIS OF THE LIVER

LILLIAN EICHELBERGER, PH.D., AND MICHAEL ROMA
CHICAGO, ILL.

WATER AND ELECTROLYTE DISTRIBUTION IN BLOOD AND TISSUE

THIS report of a case of generalized anasarca of unknown origin in an impounded dog is of special interest not only because of the anasarca but because of the presence of an extensive ascites. The purpose of this paper, therefore, is to present data on the water and electrolyte distribution in blood and tissues of an animal with an extensive gross edema of the entire body. The data obtained should indicate possibly a maximum disturbance in the equilibria of water and electrolytes of the body under conditions of gross edema and ascites.

A Boston bull terrier, male, weighing approximately 12 kilograms, was received from the pound (Fig. 1). He was unable to stand because of the extreme gross edema of the hind- and forelegs which had transformed the extremities into useless trunks because of their weight. The presence of a large volume of ascitic fluid further hindered his power to stand. Extreme pitting, soft edema was distributed throughout the body; even the face was swollen. The skin of the legs and abdominal wall oozed under the least pressure produced by slight rubbing. The subcutaneous fluid could be shifted around with a change in the position of the animal. The dog was therefore anesthetized and operated upon.

Since the subcutaneous fluid and edema obscured the femoral vein for nembutal injection, the region was cocainized, an incision then made, and the vein exposed for injection of the anesthetic. Blood was taken from the exposed femoral artery; 20 c.c. were withdrawn under oil for the serum analyses, and 10 c.c. were defibrinated for whole blood analyses. After blood and tissue had been removed, the dog was killed.

Autopsy showed that the dog had not eaten lately, for the stomach and the pale edematous gut were empty. The bladder also was empty. The peritoneal cavity contained two liters of ascitic fluid; fluid was present in the pleural cavity. The heart and kidneys seemed normal in size. There was no endocarditis. The liver was enlarged and cirrhotic; adhesions to the surrounding viscera were not present. There was an increased firmness and a gross nodularity of the tawny brown surface.

The microscopic changes* are summarized as follows.

Liver.—The sections of the liver showed large areas of atrophy and disappearance of liver cells with the blood sinuses forming alveolar blood spaces. These areas ran across many lobules, usually sparing the periportal region and not definitely related to the central veins. Around these areas were masses of pigment-filled cells with some leucocytic infiltration. The

From the Departments of Medicine and Biochemistry, the University of Chicago.
Received for publication, April 5, 1946.
*Kindly done by Dr. Louis Leiter.

portal areas in this part of the liver showed distention of bile ducts with mucus, degeneration of the epithelium, and considerable infiltration with inflammatory cells. In the areas of hemorrhagic necrosis there was marked increase in connective tissue. The whole picture suggested an infection of the bile ducts with some obstruction, secondary fibrosis, and a considerable destruction of liver parenchyma which eventually would have ended in a severe biliary cirrhosis.

Kidneys.—The sections showed relatively large glomeruli with thick framework or basement membrane with moderate increase in fibrous tissue in some but no evidence of chronic inflammation or acute change. There were no large scars and no protein in the glomerular spaces. The tubules showed considerable degeneration with protein and colloid droplets in the lumen of the convoluted tubules and a few casts in the medullary region. No calcification and no interstitial inflammation nor remarkable changes were seen in the blood vessels. There was no evidence of prolonged tubular disease with atrophy and secondary fibrosis. All in all, the kidneys might be considered normal for an old dog, except for the tubular degeneration which could be a terminal event.

Heart.—The sections showed no interstitial edema, no evidence of inflammation, no remarkable increase in connective tissue, and no vascular changes.

Skin.—The sections showed a massive edema of the entire thickness with swelling of the collagen bundles of the corium and new capillary formation in the loose subepidermal zone and also in the deeper layers of the skin. There was no evidence of inflammation or infection. The small arteries showed thick fibrous media which might indicate the age of the dog.

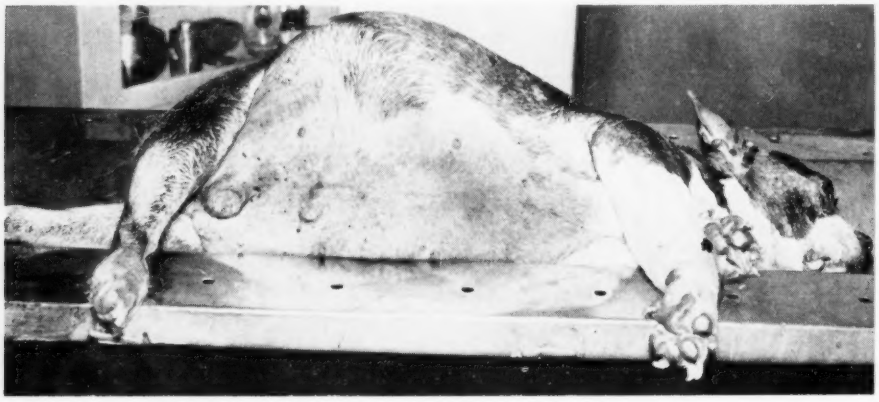

Fig. 1.—Impounded dog showing ascites and generalized anasarca.

PROCEDURES AND METHODS

The physiologic procedures and chemical methods used here were the same as those employed in previous studies on blood,[1, 2] skeletal muscle,[2, 3] skin,[4] and brain.[5] The following determinations were made: on the ascitic fluid, water, chloride, sodium, potassium, calcium, magnesium, total nitrogen, and NPN; on the serum, water, chloride, sodium, potassium, calcium, magnesium, NPN, total protein, and A/G ratio; on the whole blood, water, chloride, sodium, potassium, and cell volume; on the skin, fat, water, chloride, sodium, potassium, calcium, magnesium, and total nitrogen; on skeletal muscle, fat, water, chloride, sodium, potassium, calcium, magnesium, and content of blood; on the cerebellum and cerebral hemispheres, water, chloride, sodium, potassium, and total nitrogen.

All ascitic fluid, serum, and blood analyses were made in duplicate. The composition of the red blood cells was estimated from the analyses of the whole blood and serum and the accompanying measurement of cell volume. All tissues analyses were made either in duplicate or quadruplicate.

The analytic data on muscle were subjected to the same method of calculation as that used previously[2] to estimate phase volumes of skeletal muscle. Briefly, $(F = \frac{(Cl)_M \times (H_2O)_S \times 1000}{1.04 \times (Cl)_S}$ in which (F) represents the amount of extracellular phase per kilogram of muscle, and the subscripts M and S represent muscle and serum, respectively. From the value for (F) the intracellular phase (C) of 1 kg. of muscle was estimated: (C) = 100 - (F). From the value for (C) the intracellular water $(H_2O)_C$ was estimated: $(H_2O)_C = (C) - (S)$, in which S represents solids per kilogram of muscle.

The tissues were removed from the animal as follows: The chest and abdomen were shaved, and the skin was washed with distilled water and dried with gauze. A section of skin approximately 8 by 10 cm. was dissected from the ventral wall of the abdomen and chest and placed in a glass stoppered weighing bottle. One of the rectus abdominis muscles was then removed. Although these tissues were handled with the greatest of care, some fluid was lost from them as they were being removed and quickly placed in glass stoppered weighing bottles. The removed skin and skeletal muscle were immediately placed on a tile and trimmed quickly to remove all visible connective tissue, blood vessels, and fat. The muscle was returned to the weighing bottle and minced with scissors, and aliquot parts were weighed for all analyses. The skin was cut into small strips approximately from 1.5 to 2 mm. and placed in a weighed, glass-stoppered weighing bottle and, after being weighed, was placed in an oven at 102° C. and dried to a constant weight. The dried skin was then extracted for neutral fat, after which the strips of skin were transferred quantitatively to a special apparatus and pulverized. The pulverized mixture was returned to a glass stoppered weighing bottle, and aliquots were weighed for all analyses.

The brain was removed after the muscle and most of the bone had been taken from the skull. The brain stem and cerebellum, after separation by section through the peduncles at the level of the superior colliculi, and also the cerebral hemispheres were placed in glass stoppered weighing bottles. The tissues were minced with scissors, and weighed aliquots were used for all analyses.

RESULTS

Analytical Data on Blood and Tissues

Values for Constituents of Serum, Ascitic Fluid, and Blood Cells.—In Table I are presented the analyses of the serum, ascitic fluid, and blood cells from the dog with generalized anasarca and from normal dogs, the normal averages being included for comparison. When the findings are compared, the greatest differences are found in the water, protein, potassium, calcium, and

TABLE I. ANALYSES OF ASCITIC FLUID, BLOOD SERUM, AND CELLS FROM NORMAL DOGS AND DOG WITH ANASARCA

	H_2O (GM.)	Cl (MEQ.)	Na (MEQ.)	K (MEQ.)	Ca (MEQ.)	Mg (MEQ.)	NPN (MG.)	TOTAL PROTEIN (GM.)	CELL VOLUME (C.C. PER 100 C.C.)	ALBUMIN (GM.)
Normal dogs*										
Serum	922.0	107.1	141.2	3.95	4.94	1.73	285.0	58.5		
σ†	5.1	2.3	3.5	0.40	0.14	0.24	22.0	3.1		
Cells	703.0	62.3	93.9	9.37					46.8	
σ†		6.4	7.7	1.1					5.4	
Anasarca dog										
Serum	39.6	109.7	140.2	5.70	4.06	5.24	758.0	42.2		27.2
Cells	744.0	64.8	91.4	6.89					36.0	
Ascitic fluid	956.3	112.5	138.8	5.88	3.60	1.80	662.0	12.0		

The concentrations are expressed per kilogram of fluid and serum and per liter of red cells.

*See Reference 6.

†Standard deviation.

magnesium content of the serum and the water content of the cells. The water content of 93.96 per cent in the serum was the result of an osmotic adjustment following the lowering of the protein concentration to 4.22 per cent. The lowered serum calcium value of 4.0 meq. per kilogram occurred in response to the lowered protein concentrations in order to maintain a normal calcium ion concentration. The large increase in magnesium is spectacular. It may reflect soft tissue breakdown and renal excretory impairment.

There was a moderately low hematocrit value of 36 per cent, which might be explained as a result of existing hemodilution.

The ascitic fluid showed a protein content of 1.2 Gm. per cent. This value for protein content indicates that there has been little alteration in the permeability of the capillaries or else a considerable protein content would have been obtained. Since the ascitic fluid usually shows more protein than does the subcutaneous edema, the general edema fluids must have contained less than one per cent of protein.

TABLE II. ANALYSES OF TISSUES FROM NORMAL DOGS AND DOG WITH ANASARCA

	H_2O (GM.)	Cl (MEQ.)	Na (MEQ.)	K (MEQ.)	Ca (MEQ.)	Mg (MEQ.)	TOTAL NITROGEN (GM.)
Normal dogs							
Muscle*	775.0	18.41	23.07	98.4	1.63	18.11	
a	8.6	3.6	4.20	7.5	0.35	1.87	
Skin†	708.3	86.7	96.5	22.46	3.01	3.03	46.8
a	20.1	2.5	4.2	2.7	0.52	0.37	3.7
Cerebellum‡	745.0	35.19	50.8	92.7	2.14	10.80	19.1
a	7.0	0.89	1.7	4.0	0.14	0.60	0.5
Hemisphere‡	761.3	36.71	51.0	95.6	2.14	11.26	18.9
a	8.3	1.05	2.4	4.7	0.14	1.12	0.3
Anasarca dog							
Muscle*	816.0	45.45	55.8	81.8	2.8	11.12	
Skin†	866.6	104.0	84.9	16.1	4.54	3.74	21.6
Cerebellum‡	742.2	38.1	56.6	88.4			19.3
Hemisphere‡	768.8	39.0	55.7	87.5			18.5

The concentrations are expressed in units per kilogram.
*Units are expressed per kilogram of fat-free, blood-free muscle.
†Units are expressed per kilogram of fat-free skin.
‡Units are expressed per kilogram of wet brain.

Values for Constituents in Tissues.—In Table II are presented the analyses of skin, skeletal muscle, and brain from the edematous animal and from normal dogs. In Table III are presented the values expressed in units per 100 Gm. solid. When the tissue findings from the edematous dog are compared with those from normal dogs, the greatest differences are found in the skin and muscle values. The brain tissues, cerebellum, and cerebral hemispheres demonstrated no change in total water content and only small increases in chloride and sodium concentrations. Relative increases are shown in the total water of 15.8 per cent in skin and 4.1 per cent in skeletal muscle; in the chloride of 17.3 meq. per kilogram of skin and 27.04 meq. per kilogram of muscle; and in the sodium of 32.7 meq. per kilogram of muscle. The potassium was 6.3 meq. lower in skeletal muscle than in the normal animals. These findings on skin and muscle therefore suggest an enormous extracellular phase volume accompanied by a lowered intracellular phase volume.

TABLE III. TISSUE ANALYSES OF NORMAL DOGS AND ANASARCA DOG

	H_2O (GM.)	cl (MEQ.)	Na (MEQ.)	K (MEQ.)	Ca (MEQ.)	Mg (MEQ.)	TOTAL NITROGEN (GM.)
Skin							
Normal	243	29.7	33.1	7.71	2.06	2.08	16.0
Anasarca	652	78.0	63.6	12.08	1.70	1.40	16.2
Muscle							
Normal	344	8.2	10.2	43.8	0.73	8.05	
Anasarca	444	24.7	30.3	44.5	1.52	6.05	
Cerebellum							
Normal	292	13.8	19.9	36.3			7.5
Anasarca	288	14.8	21.9	34.3			7.5
Hemisphere							
Normal	328	15.7	21.8	40.9			8.1
Anasarca	333	16.9	24.2	37.9			8.0

All values are expressed in units per 100 Gm. solid.

DISCUSSION

Although it is almost impossible to decide the exact etiologic factors in this dog's edema, it is possible to make a few tentative speculations.

The total serum protein concentration of 4.22 Gm. per cent, of which 2.72 Gm. was albumin and 1.50 Gm. was globulin, could have been one of the causal factors. The serum proteins were reduced to a level where the colloid osmotic pressure of the plasma was not sufficient to counterbalance the hydrostatic pressure in the capillaries. This tends to force fluid out of the blood into the tissue spaces. Similar filtration types of edema with low serum protein content have been observed in nephrotic types of edema, in malnutrition, and in every disturbance of the formation of serum proteins.[7]

Whether the low protein values were the result of a loss of proteins in the urine or malnutrition or a deficient generation resulting from disease, or a combination of all these factors cannot be stated Albuminuria, for which we have no direct evidence, could not have been responsible with the serum albumin still 2.72 Gm. per cent. Furthermore, the serum cholesterol content of 192 mg. per cent suggests the absence of tissue starvation.

The anatomic changes in the liver suggest that the severe liver disease may have been sufficient to have interfered with the formation of the serum proteins and so accounted for the hypoalbuminemia. Zeldis and Alling[8] have given evidence to suggest a retarded rate of restoration of albumin in association with cholangitis and hepatitis in a dog.

It is interesting that the level of the serum albumin in this dog with cirrhosis of the liver and ascites is similar to those presented on many patients with cirrhosis of the liver. Post and Patek[9] have reviewed the extensive literature and have suggested that although the reduction in serum albumin is the essential factor in the formation of ascites it also seems likely that hypertension of the portal vein must determine the site at which the transfer of fluid takes place.

It seems unlikely that the albumin value of 2.72 Gm. per cent in the serum of this animal is low enough to cause the massive edema. The blood protein changes could have been secondary to the loss of protein in the ascitic fluid and the edema fluid. Therefore, there must have been other factors in the dog's

edema. The possibility of heart failure with increase in venous pressure, aggravated in this case in the portal area by the changes in the liver, must be considered.

The histologic changes in the kidney do not indicate that the edema was the result of organic renal excretory insufficiency. Without a doubt the nonprotein nitrogen retention (76.8 mg. per cent) in the serum was masked to a large degree by the dilution of waste products in the edema fluid. In view of the relatively well-preserved kidneys one must assume prerenal or circulatory azotemia with oliguria. The latter would aggravate any underlying tendency to edema.

Although we have attempted to find the cause of the generalized anasarca in a vagrant, impounded dog, the purpose of the study was to present the data on the water, protein, and electrolyte distribution in blood and tissues. These data might be considered a maximum disturbance in the equilibria of water, protein, and electrolytes resulting from gross edema and ascites.

The view that the skin is a great reservoir capable of taking care of large volumes of water[4, 10] is again demonstrated here. Skin under normal conditions contains an average of 70.83 per cent of water. In this dog there was rise to 86.66 per cent, which is actually 22.2 per cent higher than the normal skin value. The distribution of this water in skin cannot be definitely ascribed, but the tremendous increases in sodium and chloride concentrations indicate that the large increase is in the extracellular phase. In skeletal muscle the total water content was 81.60 per cent, or 4.1 Gm. of water higher than the muscle removed from normal dogs. This actually represents a 5.3 percentage increase in water. If the distribution of this water is computed (muscle, anasarca, Table IV), the extracellular phase (F) is 375 Gm. or a 150 per cent increase over the value for a kilogram of normal dog muscle. Some of this increase was the result of a transference of water from the intracellular phase, since the volume of intracellular water $(H_2O)_C$ per kilogram was estimated to be 441 Gm., the normal value being 620 Gm.

To estimate the absolute changes in volume which the normal muscle has undergone during the course of disease, we assume that the muscle before the

TABLE IV. PHASE VOLUME DATA ON MUSCLE FROM ANASARCA DOG

	M	(F)	$(H_2O)_C$	(S)	$\{H_2O\}_C$
Muscle					
Normal*	1000	155	620	225	733
Anasarca	1000	375	441	184	
$(M)_f$	1224	459	540	225	705
Δ	+224	+304	−80		−28

All values are expressed in grams per kilogram of fat-free, blood-free muscle.

M = Extracellular phase and intracellular phase.

(F) = Grams of extracellular phase per kilogram of muscle.

$(H_2O)_C$ = Grams of intracellular water per kilogram of muscle.

$\{H_2O\}_C$ = Grams of water per kilogram of muscle cells.

(S) = Solids of intracellular phase.

$(M)_f$ = Absolute final weight of the initial kilogram of normal muscle after the effects of the disease.

Δ = Differences between values obtained on normal muscle and the absolute values following the effects of the disease $(M)_f$.

*It is assumed that the initial muscle is the normal muscle of the dog before the onset of disease.

onset of disease was a normal muscle possessing normal volume values (normal muscle, Table IV). Also, we assume that during the disease there has been no alteration in the amount of solids present in that quantity of muscle cells in a kilogram of normal muscle—(S) normal muscle, Table IV. In other words, if the cell solids of a kilogram of normal muscle $(S)_N$ is divided by the cell solids of a kilogram of muscle as removed from the anasarca dog $(S)_A$, a factor is obtained to multiply all volume values of the anasarca muscle to obtain the absolute values—$(M)_f$, Table IV. The absolute values so obtained showed that an *increase* of 224 Gm. per kilogram of control muscle (M) had occurred, of which a 304 Gm. increase were attributed to the extracellular phase (F) and a *loss* of 80 Gm. (C) from the muscle cells. Actually, the extracellular phase volume of the original kilogram of muscle is now 459 Gm. Thus, in this animal the edema of the skeletal muscle is all extracellular and is accompanied by a dehydration or shrinkage of the muscle cells. As a result the percentage of water per kilogram of muscle cells $(H_2O)_C$ is now 70.5 per cent, instead of the normal value of 73.3 per cent (Table IV).

In spite of the large volumes of extracellular fluid present in the skin and skeletal muscle, neither the cerebellum nor the cerebral hemisphere had increases in total water content. There were, however, small changes in the chloride and sodium content, which indicated small increase in the extracellular phase at the expense of the brain cells. This finding demonstrates the constancy with which the fluid of the brain is distributed even when the interstitial spaces of all other tissues of the body are filled with extracellular edema.

SUMMARY

Data on the water, protein, and electrolyte distribution in blood and tissues of an impounded dog with cholangitis, early biliary cirrhosis, and generalized anasarca are presented.

By analyses the total serum protein concentration of 4.22 Gm. per cent, of which 2.72 Gm. per cent was albumin and 1.5 Gm. per cent was globulin, could have been an important causal factor in the edema of the dog, but other factors must have been present.

With the presence of this type of edema the skin and muscle had increases in water content as much as 22.2 and 5.3 per cent, respectively. The large increases in sodium and chloride concentrations in the skin indicate that the increased water content was extracellular. The total bulk of a kilogram of muscle before the onset of disease was calculated to have *increased* during the disease 224 Gm. or to a total volume of 1,224 Gm., of which a 304 Gm. *increase* were attributed to the extracellular phase and a *loss* of 80 Gm. from the muscle cells. This is an example of edema and dehydration appearing simultaneously. In spite of the extracellular edema existing in skin and muscle, neither the cerebellum nor cerebral hemispheres showed any increase in total water content. This finding demonstrates the constancy with which the fluid of the brain is distributed even when the interstitial spaces of all other tissues of the body are filled with extracellular edema.

REFERENCES

1. Eichelberger, L., and Roma, M.: Experimental Hydronephrosis in Dogs, J. Urol. **40**: 366-377, 1938.
2. Hastings, A. B., and Eichelberger, L.: The Exchange of Salt and Water Between Muscle and Blood, J. Biol Chem. **117**: 73-93, 1937.
3. Eichelberger, L.: The Distribution of Body Water in Skeletal Muscle in Dogs With Impaired Renal Function, J. Biol. Chem. **128**: 137-152, 1939.
4. Eichelberger, L., Eisele, C. W., and Wertzler, D.: The Distribution of Water, Nitrogen and Electrolytes, J. Biol. Chem. **151**: 177-189, 1943.
5. Eichelberger, L., and Richter, R. B.: Water, Nitrogen and Electrolyte Concentration in Brain, J. Biol. Chem. **154**: 21-29, 1944.
6. Eichelberger, L., and Roma, M.: The Distribution of Body Water and Electrolytes in Skeletal Muscle of Dogs With Experimental Hydronephrosis Following Injection of Potassium Salts, J. Biol. Chem. **140**: 467-481, 1941.
7. Leiter, L.: Nephrosis, Medicine **10**: 135-242, 1931.
8. Zeldis, L. J., and Alling, E. L.: Plasma Protein Metabolism—Electrophoretic Studies, J. Exper. Med. **81**: 515-537, 1945.
9. Post, J., and Patek, A. J.: Serum Proteins in Cirrhosis of the Liver, Arch. Int. Med. Med. **69**: 67-82, 1942.
10. Skelton, H.: The Storage of Water by Various Tissues of the Body, Arch. Int. Med. **40**: 140-152, 1927.

SENSITIZATION OF Rh-POSITIVE PATIENTS BY THE Rh FACTOR

LESTER J. UNGER, M.A., M.D.
NEW YORK, N. Y.

THE discovery of the Rh factor and the recognition of the role that it plays in connection with transfusion have created certain practical problems. The fact is now recognized that when repeated transfusions of Rh-positive blood are given to an Rh-negative patient antibody response to the Rh antigen develops in approximately one in twenty-five patients. Instances in which antibodies develop after one transfusion also have been reported.[1] These antibodies may be bivalent (anti-Rh agglutinins) or univalent (blocking antibodies).[2] Further transfusions of Rh-positive blood into patients so sensitized result in hemolytic reactions. Approximately 90 per cent of all hemolytic posttransfusion reactions are caused by the Rh factor. It is also established that exposure of an Rh-negative woman of any age, from birth to the climacteric, to Rh-positive blood by transfusion may result in her inability to give birth to a normal infant. Furthermore, repeated pregnancies in an Rh-negative woman bearing Rh-positive children may sensitize the patient, even though clinically all of her children may have been apparently normal. In such a patient transfusion may be followed by a hemolytic reaction.

It is apparent that it becomes incumbent upon everyone ordering transfusions to understand the mechanism involved and to avoid the development of posttransfusion hemolytic reactions. All who select donors and perform tests necessary for the selection of a donor must revise their technique in the light of recent advances. A previous paper[3] referred to various routines now followed. It was stated that the ideal procedure is to perform an Rh typing on every patient, as well as on every donor, and to use for transfusion only a donor belonging to the same Rh type as the patient, as well as one of the same ABO group. It was pointed out that perhaps in the future, and it is hoped in the not distant future, this will be the accepted technique. For the present it was advised that the blood of every patient be tested for the Rh_o factor, that all those who react positively to this factor (85 per cent) be classified as Rh-positive patients, and that all those who react negatively be classified as Rh-negative patients. It was recommended also that the bloods of all donors be tested with Rh_o' serum and all those who react positively (87 per cent) be classified as Rh-positive donors, while those who react negatively be classified as Rh-negative donors. For transfusion of all patients classified as Rh positive Rh-positive donors should be used; for all Rh-negative patients the blood of Rh-negative donors should be selected. This routine will eliminate from the roster of Rh-negative donors all individuals who are positive either to the Rh_o or the Rh′ factor. This routine, however, deliberately disregards donors belonging

From the Blood and Plasma Division, New York Post-Graduate Medical School and Hospital.
Received for publication, May 1, 1946.

to type Rh″, and they would be included in the roster as Rh negative. Such donors are rare, constituting only 0.6 per cent of the total, while Rh-negative patients constitute approximately 15 per cent of the total patients. Such a combination, namely an Rh″ donor (classified as Rh negative) and used for the transfusion of an Rh-negative patient with subsequent development of anti-Rh″ agglutinins, is very unlikely. On the other hand this idea fails to take into consideration the fact that when blood of Rh-positive donors is transfused into Rh-positive patients 50 per cent of all patients are Rh positive and belong to Type Rh_1. They lack the Rh″ factor just as do Rh-negative patients. The blood of approximately 28 per cent of all donors belongs to types Rh_2 and Rh_1Rh_2 and contains the Rh″ factor. Exactly how frequently sensitization results from such a combination remains to be seen; it probably is not common. The possibility exists, however, that it occurs more often than we now suspect and that the cases go unrecognized. This opinion is based on the fact that at present if Rh determinations are done on patients and donors prior to transfusion the test is merely for the Rh_o factor. If both patient and donor are classified as Rh positive, and a posttransfusion reaction occurs, the reaction is considered pyrogenic in nature; thus, the diagnosis of a hemolytic reaction is dismissed, especially if jaundice or hemoglobinuria do not develop. Yet these clinical symptoms are not always present, and the diagnosis can be made only serologically. The following case illustrates the fact that Rh-positive blood transfused into an Rh-positive patient can cause sensitization, and a hemolytic posttransfusion reaction can result. This is the first completely proved case of Rh″ sensitization induced by transfusion in an Rh-positive patient; demonstrable antibodies were found in the patient's serum.

CASE REPORT

C. M., a white man, 25 years of age, was referred to me by Colonel E. S. Olson and Captain F. E. Fonseca, of the Medical Corps. While on army duty in India in 1942 the patient fell from a troop train and sustained compression fractures of the ninth dorsal and first lumbar vertebrae, injuring the spinal cord. He developed a spastic paraplegia. Because of involuntary micturition and defecation a suprapubic cystostomy and a perineal urethrotomy were done. In 1943, a laminectomy and exploration at the site of the spinal injury was performed but was not followed by any significant improvement. For the past two years he has been suffering from numerous decubitus ulcers which necessitated vigorous supportive therapy, especially in the form of repeated blood transfusions. The patient's blood was classified as Group AB, Rh positive. The first transfusion was given Oct. 15, 1944, and from then to March 28, 1946, or during a period of approximately one year and four months, he received a total of twenty-three blood transfusions and one plasma transfusion. On one occasion the transfusion was of 1,000 c.c., but on each other occasion 500 c.c. were given. For the first twenty transfusions Rh-positive blood was transfused.

In Table I is given the patient's transfusion history. Because of the reactions that followed the transfusions, and because these reactions were becoming more severe, I was asked to see the patient. Examination of his blood showed it to be Group AB, Subgroup A_2, type M, Rh_1, Hr positive. Examination of the patient's serum (Table II) showed the presence of anti-Rh″ agglutinins in a titer of 1:4. Anti-Rh antibodies of other types were not and,

TABLE I. TRANSFUSION HISTORY OF PATIENT (GROUP AB INTERNATIONAL)

DATE OF TRANSFUSION	Rh DETERMINATION OR TYPE	AMOUNT TRANSFUSED	POSTTRANSFUSION REACTION
11/15/44	Positive	500 c.c. blood	Urticaria
8/ 1/45	Positive	500 c.c. blood	No reaction
9/ 4/45	Positive	500 c.c. blood	No reaction
9/13/45	Positive	500 c.c. blood	Urticaria
9/19/45	Positive	500 c.c. blood	No reaction
10/ 3/45	Rh_2	500 c.c. blood	Chill, cyanosis, urticaria
10/18/45	Rh_1	500 c.c. blood	No reaction
10/ 3/45	Positive	500 c.c. blood	No reaction
11/21/45	Positive	500 c.c. blood	Temperature 103° F., mild urticaria
11/29/45	Positive	500 c.c. blood	Chill, temperature 102° F.
1/ 3/46	Positive	500 c.c. blood	No reaction
1/19/46	Positive	500 c.c. blood	No reaction
2/ 6/46	Rh_1	500 c.c. blood	No reaction
2/ 8/46	Positive	500 c.c. blood	No reaction
2/11/46	Positive	500 c.c. blood	No reaction
2/13/46	Positive	500 c.c. blood	Chill, temperature 103° F.
3/11/46	Positive	500 c.c. blood	Temperature 102° F.
3/13/46	Positive	500 c.c. blood	Chill, temperature 104° F., urticaria
3/15/46	Positive	500 c.c. blood	Chill, temperature 105° F., urticaria
3/18/46	Positive	500 c.c. blood	Chill, temperature 104° F., urticaria
3/19/46	Positive	500 c.c. plasma	Urticaria
3/21/46	Negative	1,000 c.c. blood	Urticaria
3/22/46	Negative	500 c.c. blood	No reaction
3/28/46	Negative	500 c.c. blood	No reaction

TABLE II. TITRATION OF ABNORMAL ANTIBODIES IN SERUM OF PATIENT REPORTED

DATE OF TEST	TEST CELLS	DILUTION OF PATIENT'S SERUM				
		UNDILUTED	1:2	1:4	1:8	1:16
3/19/46 Tested by agglutination method	rh*	−	---	---	---	---
	Rh_o	−	---	---	---	---
	Rh_1	---	---	---	---	---
	Rh″	++	+	+	---	---
	Rh_2	++	+	+	---	---
3/19/46 Tested by conglutination method	rh	---	---	---	---	---
	Rh_o	---	---	---	---	---
	Rh_1	---	---	---	---	---
	Rh″	++±	+±	±	tr	---
	Rh_2	++	+	tr	---	---
3/27/46 Tested by agglutination method	rh	---	---	---	---	---
	Rh_1	---	---	---	---	---
	Rh_2	++	+±	+	tr	---
3/27/46 Tested by conglutination method	rh	---	---	---	---	---
	Rh_1	---	---	---	---	---
	Rh_2	++	+±	+	±	---

Tests were also made at other times, but the results are not given because they were essentially the same.

*Type rh is the short designation for Rh-negative blood.

of course, could not be present. No Rh_o blocking antibodies were present. By the conglutination method performed in the test tube, tests also demonstrated the Rh″ specificity; the result was positive in a titer of 1:4. When tested

on the slide the reaction also was positive only against blood containing the Rh″ factor. It will be noted from Table I that the blood used for the first twenty transfusions was in each instance Rh positive. Ten of these were followed by no reaction whatsoever. Two were followed by urticaria, one by fever and urticaria, two by chill and fever, and four by chill, fever, and urticaria. The urticarial reaction was undoubtedly due to protein sensitization as evidenced by the fact that urticaria also developed after the administration of 500 c.c. of plasma. The six transfusions which were followed by a chill were undoubtedly hemolytic in nature. It is possible here to report the result of the examination of the blood of only three of the donors used. One belonged to type Rh_2 and transfusion of his blood gave the most severe reaction; two donors belonged to type Rh_1, and the transfusions of their bloods were followed by no reaction whatsoever. These results coincided with the fact that the patient's blood belonged to type Rh_1, and his serum contained Rh″ agglutinins. One or more of the first five donors had blood containing the Rh″ factor. This was the only factor lacking in the patient's blood; he became sensitized and developed anti-Rh″ agglutinins. The blood of the sixth donor belonged to type Rh_2, and its administration was followed by a severe hemolytic reaction. From then on every time blood containing the Rh″ was given a hemolytic reaction resulted. Every time blood, even though Rh positive, was of a type lacking the Rh″ factor no reaction followed.

This particular case is complicated by the fact that there was a protein sensitization as evidenced by the appearance of urticaria on five occasions. On one occasion this symptom followed the administration of plasma, which, of course, contained no red blood cells. After the blood was examined, and the fact that it contained anti-Rh″ agglutinins was discovered, only Rh-negative blood was used. Three such transfusions were given without hemolytic reaction. One of these transfusions consisted of 1,000 c.c. (500 c.c. of washed Rh-negative Group O cells and 500 c.c. Rh-negative Group AB whole blood). This was followed by the appearance of a few urticarial lesions but no other untoward symptoms.

It should be pointed out, however, that it is unnecessary to use Rh-negative blood for a patient such as this. In fact such procedure is uneconomical and depletes the bank of Rh-negative blood. In order to have Rh-negative blood available when it is actually needed, it should be used only for Rh-negative patients. Inasmuch as this patient belonged to type Rh_1 a donor belonging to type Rh_1 should be used. The supply of Rh_1 blood is plentiful because such donors constitute 50 per cent of the total, whereas Rh-negative donors constitute only 15 per cent.

This case illustrates that sensitization by the Rh″ factor can occur and does occur in Rh-positive patients belonging to type Rh_1, and that simply using Rh-positive blood donors for Rh-positive patients is not complete protection. To avoid this, one must carry out either one of two routines. The ideal procedure is to perform a complete Rh typing on every patient and only use blood of the same Rh type, or at least not to administer blood which contains an Rh factor lacking in the patient. An alternate and simpler routine

is to use the appearance of a posttransfusion reaction as a signal for careful investigation of the patient's blood. The first reaction is never, or hardly ever, fatal. Subsequent ones may be, although in this case the patient survived six hemolytic reactions. These reactions were most distressing and were becoming progressively more severe and more serious. If after the transfusion given Oct. 3, 1945, when the patient developed a chill and cyanosis, examinations had been performed, it would have become evident that the patient belonged to type Rh_1 and the donor to Rh_2, and the Rh″ agglutinins would have been detected. The next donor was Rh_1, and in all likelihood the one used following this was Rh_1 because no reaction occurred. The next two were followed by reactions, undoubtedly because the blood contained the Rh″ factor. It is persistence in giving transfusions in the presence of a reaction that leads to trouble and in some instances to the death of the patient. Fortunately this did not occur in this instance. For the average institution which lacks the necessary sera and the personnel to perform these tests, this alternative and simpler rule is recommended: namely, *as soon as a posttransfusion reaction occurs, give no further transfusion until a complete investigation has been made*. Such blood, if it cannot be examined at the institution where the patient is hospitalized, should be sent to those laboratories which specialize in this field. There is, however, a simple test which is helpful and should be used except in women prior to the climacteric.[4] Prior to the transfusion a specimen of the patient's blood should be drawn in 3 per cent sodium citrate solution (one part citrate solution, nine parts blood). This specimen can be used for the customary typing and cross-matching tests. If a reaction occurs, a second similar specimen should be drawn during the reaction or shortly after it has subsided. These two specimens should then be centrifuged and the color of the supernatant plasma compared. If the color before and after transfusion is identical, the reaction was pyrogenic and not hemolytic. If on the other hand the color of the plasma of the posttransfusion specimen is deeper than that of the pretransfusion specimen, the reaction is hemolytic. No further blood should be transfused until the problem is clarified and the proper blood obtained.

SUMMARY

The Rh factor is responsible for approximately 90 per cent of all hemolytic posttransfusion reactions. If only Rh-negative blood is administered to patients who are Rh negative, sensitization by the Rh factor will be avoided in such patients. In certain cases when blood is administered to Rh-positive patients, sensitization may follow providing the patient lacks any of the three Rh factors and the donor's blood contains the factor or factors absent in the blood of the patient. A case illustrating this point is reported. The patient belonged to type Rh_1 and therefore lacked the Rh″ factor. Donor's blood containing the Rh″ factor had been administered and anti-Rh″ agglutinins developed in the blood of the patient. The donor whose blood produced the first hemolytic reaction experienced in this case belonged to type Rh_2, whereas subsequent transfusions with blood belonging to type Rh_1, or Rh negative, were followed by no hemolytic reaction. In order to protect these patients complete Rh

typing must be performed, and only blood of the same Rh type administered or the appearance of a reaction must be used as an indication for complete serologic examination of the patient's blood. After the appearance of one reaction, no further transfusion should be given until serologic examination shows that the reaction was not hemolytic in nature. Clinically, patients do not benefit from transfusion followed by a hemolytic reaction. Persistence in giving transfusion in spite of a reaction may be dangerous.

REFERENCES

1. Unger, L. J.: Blood Banks of the Future With Special Reference to the Role of the Rh Factor. In press.
2. Wiener, A. S.: Rh Factors in Clinical Medicine, J. Lab. & Clin. Med. 30: 957-976, 1945.
3. Unger, L. J., Weinberg, M., and Lefkon, M.: The Rh Factor as Applied to the Operation of Blood Banks. In press.
4. Wiener, A. S.: Blood Groups and Transfusion, ed. 3, Springfield, Ill., 1943, Charles C Thomas, Publisher, p. 71.

THE TRAUMATIC EFFECTS OF POSITIVE INTRATRACHEAL PRESSURES

CAPTAIN HOWARD TATUM AND LIEUTENANT COLONEL ARTHUR M. GINZLER,
MEDICAL CORPS, ARMY OF THE UNITED STATES

THE use of positive pressure inhalational therapy has by now received considerable support. It apparently was used first as long ago as 1878 by Oertel[1] who employed 100 inspirations of air compressed to $\frac{1}{50}$ of an atmosphere excess pressure in the treatment of severe asthma. In 1897, Norton[2] reported the successful employment of positive pressure in the treatment of pulmonary edema caused by carbolic acid. Since then, Emerson,[3] Poulton and Oxon,[4] Barach,[5] and others have recommended the use of positive pressure breathing in the treatment of bronchial asthma, chronic bronchitis, emphysema, and pulmonary edema caused by left-sided cardiac failure. Christie[6] suggested that the maintenance of a positive intra-alveolar pressure throughout the respiratory cycle might diminish the tendency to pulmonary edema in patients poisoned with phosgene, and Barach[5] indicated the importance of this procedure in the prevention and treatment of pulmonary edema caused by irritant war gases, referring to a series of patients with varying degrees of pulmonary edema due to inhalation of chlorine and nitric fumes which had been so treated with considerable success.

The employment of positive intratracheal pressure is not without potential hazard to the pulmonary tissues. Lilienthal[7] noted interstitial emphysema in a patient receiving general anesthesia by insufflation when the intratracheal pressure was raised to 60 mm. of mercury. Ewald and Kobert[8] found that air under a pressure of 40 mm. of mercury caused rupture of alveoli and pneumoperitoneum in rabbits. Joannides[9] was able to force air or fixing fluid through pulmonary alveoli into the pulmonary circulation without producing apparent gross or microscopic injury to the alveolar walls, but he and Tsoulos[10] found that when intrapulmonic pressures of from 60 to 100 mm. of mercury were attained in either normal or pneumonic dogs, interstitial pulmonary emphysema, pneumothorax, pneumoperitoneum, and air embolism invariably resulted. By means of a Van Allen carotid cannula, Polak and Adams[11] also demonstrated the occurrence of air emboli in animals subjected to increased intrapulmonic pressures.

At somewhat lower levels of pressure, Marcotte and associates,[12] working with dogs, found that intrabronchial pressures of 24 mm. of mercury maintained for periods of from 16 to 110 minutes were routinely accompanied by interstitial emphysema, usually along the great vessels at the hilum of the lungs but, on two occasions, spreading along the trachea into the neck; in one

From the Clinical Research and Pathology Sections, Medical Division, Chemical Warfare Service, Edgewood Arsenal, Md.
Received for publication, May 9, 1946.

animal interstitial emphysema resulted at a pressure of only 18 mm. of mercury. They found that such emphysema occurred in cats at from 16 to 20 mm. of mercury. Similarly, Rasmussen and Adams,[13] in single acute experiments and in chronic intermittent experiments in dogs over periods of from one week to eleven months, found varying degrees of gross and microscopic emphysema and, in a few instances, air embolism and pneumothorax at pressures of from 30 to 35 mm. of mercury at the carina.

The occurrence of such effects of increased intrapulmonic pressure in normal animals raises the question whether traumatic tissue damage of even greater degree or at even lower levels of pressure might not result in lungs already the site of damage, as indicated by the presence of pulmonary edema. A comparison was therefore made between the effects of increased intratracheal pressures in normal dogs and in dogs with pulmonary edema produced by the inhalation of phosgene. Particular note was made of the pressures required to produce arterial air emboli in the two groups of dogs.

EXPERIMENTAL

One group of dogs was gassed with phosgene to permit the development of pulmonary edema prior to the remainder of the experimental procedure. After sufficient time elapsed, these and the normal control dogs were anesthetized by the intraperitoneal injection of 20 mg. of nembutal per kilogram of body weight. Each dog was then tracheotomized, and a Y tube cannula was inserted into the trachea. One arm of the cannula was attached to a source of air pressure and the other to a mercury manometer. Arterial and venous blood pressures were recorded directly from cannulae inserted into the femoral artery and vein. After intravenous administration of 100 mg. of heparin, a Van Allen cannula was inserted into the right carotid artery to permit visualization of air emboli flowing through the artery. A tambour recording pneumograph was placed around the chest. The head of some of the animals was elevated to facilitate trapping of the air emboli in the carotid cannula.

The intratracheal pressure was then increased by 10 mm. of mercury; this was maintained for a period of ten seconds. Several minutes were then allowed for return of respiration and blood pressure to normal. In this manner, the lungs were subjected to successive increments of positive intratracheal pressure for periods of ten seconds each, with intervening rest periods of several minutes. In some instances, the experiment was concluded at 30 or 50 mm. of mercury and the animal sacrificed for gross and microscopic study. In the majority, the experiments were concluded when air emboli were noted in the carotid trap, when gross interstitial emphysema was observed through the cervical incision, or upon death of the animal.

RESULTS

Normal Dogs.—With elevation of the intratracheal pressure, there occurred a drop in arterial blood pressure, an elevation of venous pressure, and cessation of respiration (Fig. 1). These effects persisted while the increase in intra-

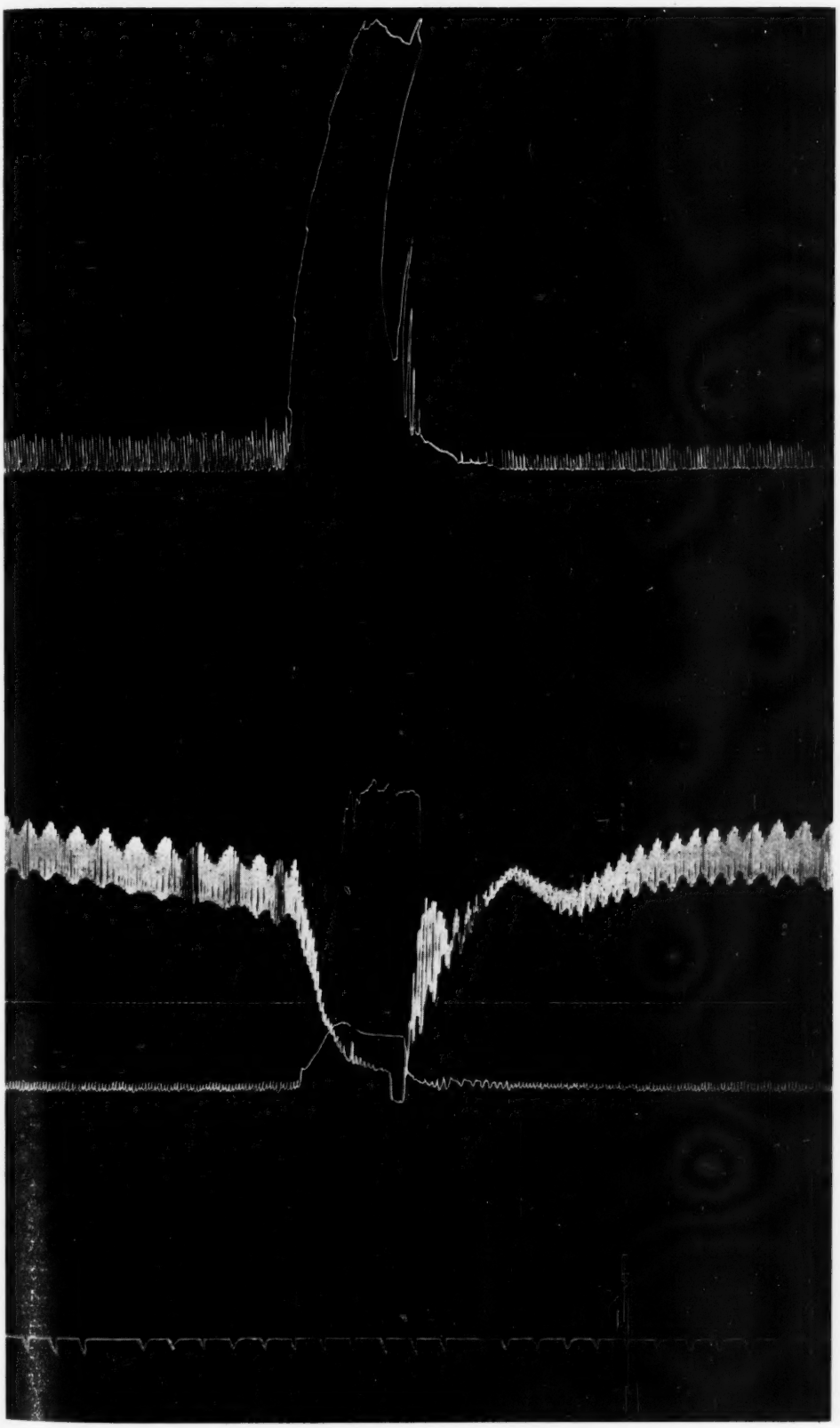

Fig. 1.—Normal dog, 60 mm. Hg intratracheal pressure.

tracheal pressure was maintained. When the increased pressure was discontinued and the intratracheal pressure allowed to drop to normal, the venous blood pressure immediately returned to normal; this was followed within a few seconds by resumption of normal respirations and a gradual return of the arterial pressure to normal level at about fifteen seconds.

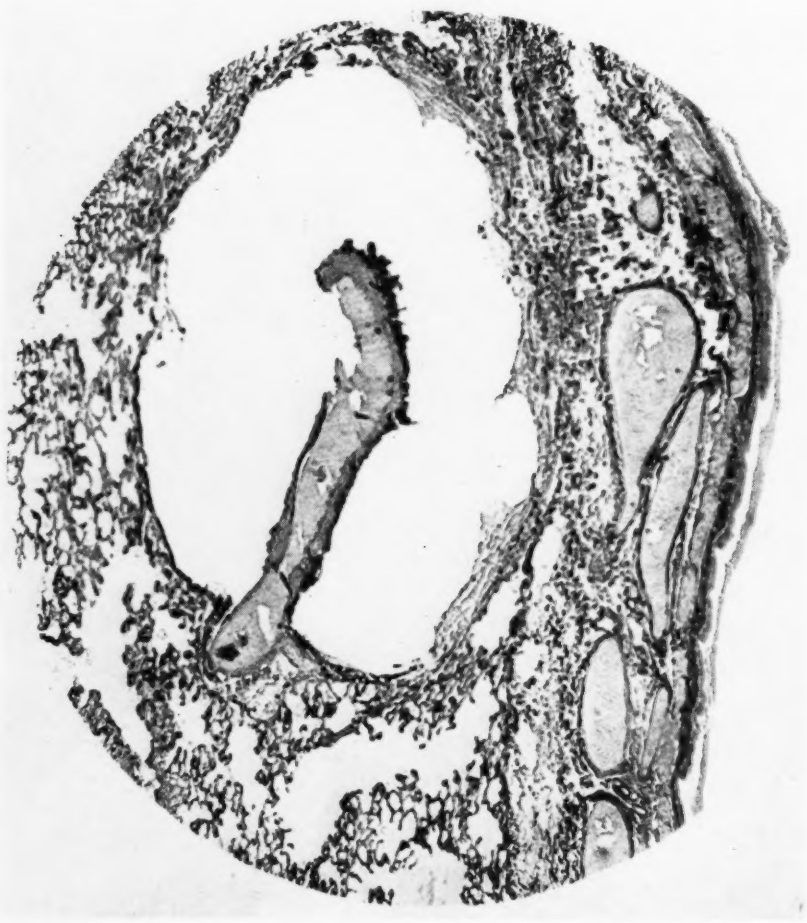

Fig. 2.—Interstitial emphysema in dog subjected to elevation of intratracheal pressure of 90 mm. of mercury. Note compression of pulmonary artery. The longitudinally cut bronchus is at the right margin. Hematoxylin and eosin (×40).

In three dogs (heads elevated), air emboli appeared in the carotid trap at increased intratracheal pressures of 80, 90, and 90 mm. of mercury, respectively. In a fourth dog (head not elevated), gross mediastinal emphysema was observed through the cervical incision at 80 mm., although no air emboli were noted in the carotid trap; in addition, autopsy revealed pneumothorax and pneumoperitoneum. Microscopic examination of the lungs of these four dogs revealed moderate dilatation of the terminal respiratory passages, often associated with focal vesicular emphysema. Only very slight, focal tearing of

alveolar septa or intra-alveolar hemorrhage was noted; in general, these were absent. The most notable feature was the presence of numerous, interstitially located bubbles of air; these were especially prominent in the larger septa toward the hilus of the lung, often associated with resulting compression of the adjacent pulmonary artery (Fig. 2). Occasionally there was associated interstitial hemorrhage.

Two dogs were sacrificed after the intratracheal pressure had been elevated to 50 mm. of mercury. No carotid air emboli had been noted in these animals, nor was there gross mediastinal or cervical interstitial emphysema. Microscopically, however, the lungs showed changes similar to, although somewhat less severe than, those in the preceding group of dogs; in particular, foci of interstitial emphysema were fairly numerous within the interlobular septa adjacent to the pulmonary arteries. One dog was sacrificed after being subjected to an increased pressure of 30 mm. of mercury; no gross or microscopic changes were observed.

Dogs With Pulmonary Edema.—In twelve dogs in which a substantial degree of pulmonary edema had developed as a result of the gassing with phosgene, the effect of increased intratracheal pressure on respiration and on the arterial and venous blood pressure was indistinguishable in type or degree from that observed in the normal dogs. In three animals of this group (heads elevated), carotid air emboli were observed at increased intratracheal pressures of 80, 110, and 110 mm. of mercury, respectively. In another five dogs (heads not elevated), one exhibited carotid air emboli at a pressure of 90 mm. of mercury, two developed gross interstitial emphysema at a pressure of 100 mm. of mercury, and the remaining two exhibited neither gross interstitial emphysema or carotid air emboli but failed to resume normal respiration upon release to normal of the intratracheal pressure which had been raised to 100 and 110 mm. of mercury, respectively. In these eight dogs, in addition to the substantial degree of pulmonary edema and other changes typical of phosgene poisoning, there were changes in the lungs attributable to the increased intrapulmonic pressure which were essentially identical with those observed in the control dogs subjected to similar levels of increased intrapulmonic pressure. It appears likely that the cause of death in the last two dogs was air embolism to the brain despite the failure of the carotid trap to visualize any air emboli.

In two dogs with pulmonary edema, increase in intratracheal pressure to 50 mm. of mercury resulted in somewhat less severe effects than in the controls at this pressure, both showing slight focal dilatation of terminal air passages and relatively few foci of interstitial emphysema within the lungs on microscopic examination. Two dogs of the group with pulmonary edema subjected to a pressure of 30 mm. of mercury showed no gross or microscopic changes in the lungs other than those caused by the phosgene.

DISCUSSION

The results observed indicate that the physiologic response of arterial and venous blood pressure and respiration to increased intratracheal pressure is essentially the same in dogs with pulmonary edema resulting from gassing

with phosgene as it is in normal dogs. Similarly, air embolism and/or gross mediastinal and cervical interstitial emphysema occur at about the same level of increased intratracheal pressure in the two groups of dogs. Both the normal dogs and those with pulmonary edema also exhibit the same degree of microscopic pathologic changes as a result of the increased pressure. The lack of significant apparent damage to the intra-alveolar septa as a result of the increased pressure is of interest; this is in agreement with the observations of Hartroft[14] in human emphysema and in substantial agreement with the findings of Rasmussen and Adams[13] in their experimental animals. Macklin[15] has inferred that, as a result of the alveolar distention, numerous fine ruptures occur in the base of alveoli abutting against the vascular sheaths rather than in the lateral alveolar walls, because of the absence of pores at the former site, and by the breaking through of air from the alveoli into the interstitial tissue in this manner explains the pathogenesis of the interstitial emphysema.

In the present study, damage was observed to occur to the lungs, both in the normal dogs and in those with pulmonary edema, at a pressure of 50 mm. of mercury but not at 30 mm. However, in view of the shortness of the bursts and in view of the findings of other investigators previously quoted, it would appear that the therapeutic use of positive pressure inhalational therapy should not exceed pressures of from 15 to 20 mm. of mercury. It would also appear that there is no greater hazard to pathologic than to normal pulmonary tissue from this procedure.

SUMMARY

1. Increased intratracheal pressure in dogs, under the conditions of this study, resulted in drop in arterial blood pressure, elevation of venous pressure, and apnea. There was no essential difference noted between the response of normal dogs and those with pulmonary edema resulting from inhalation of phosgene.

2. Air embolism and/or gross mediastinal and cervical interstitial emphysema occurred at about the same level of intratracheal pressure in the two groups of dogs.

3. The microscopic pathologic changes, other than the lesions caused by inhalation of phosgene in the one group of dogs, were also of essentially the same magnitude in the two groups of animals. These changes consisted of moderate dilatation of terminal respiratory passages, focal vesicular emphysema, and interstitial emphysema, first noted microscopically at a pressure of 50 mm. of mercury in this study. Gross mediastinal and cervical interstitial emphysema and carotid air emboli became apparent at pressures of about 80 to 110 mm. of mercury in both the control animals and in those with pulmonary edema.

4. The findings indicate that there is no greater hazard as a result of positive pressure inhalational therapy to lungs with edema than to normal lungs.

Acknowledgment is made to Mrs. Harriet Kriete, for assistance in gassing the dogs with phosgene.

REFERENCES

1. Oertel: Cited by Barach.[5]
2. Norton, N. R.: Forced Respiration in a Case of Carbolic Acid Poisoning, Med. & Surg. Rep. Presbyterian Hosp., N. Y. **2**: 321, 1897.
3. Emerson, H.: Artificial Respiration in the Treatment of Edema of the Lungs, Arch. Int. Med. **3**: 368, 1909.
4. Poulton, E. P., and Oxon, D. M.: Left Sided Heart Failure With Pulmonary Edema; Its Treatment With the Pulmonary Plus-Pressure Machine, Lancet **231**: 981, 1936.
5. Barach, A. L.: Principles and Practices of Inhalational Therapy, Philadelphia, 1944, J. B. Lippincott Company.
6. Christie, R. V.: Discussion on the Effects of Asphyxiating Gases on the Respiratory System, Proc. Roy. Soc. Med. **30**: 1033, 1937.
7. Lilienthal, H.: Thoracic Surgery, Philadelphia, 1926, W. B. Saunders Company. Cited by Joannides and Tsoulos.[10]
8. Ewald, J. R., and Kobert, R.: Arch. f. d. ges. Physiol. **31**: 160, 1883. Cited by Schwerma and Ivy.[16]
9. Joannides, M.: Insufflation of Compressed Air in Treatment for Pneumonia, Arch. Int. Med. **47**: 196, 1931.
10. Joannides, M., and Tsoulos, G. D.: The Etiology of Interstitial and Mediastinal Emphysema, Arch. Surg. **21**: 333, 1930.
11. Polak, B., and Adams, H.: Traumatic Air Embolism in Submarine Escape Training, U. S. Nav. M. Bull. **30**: 165, 1932.
12. Marcotte, R. J., Phillips, F. J., Adams, W. E., and Livingstone, H.: Differential Intrabronchial Pressures and Mediastinal Emphysema, J. Thoracic Surg. **9**: 346, 1940.
13. Rasmussen, R. A., and Adams, W. E.: Experimental Production of Emphysema, Arch. Int. Med. **70**: 379, 1942.
14. Hartroft, W. S.: The Microscopic Diagnosis of Pulmonary Emphysema, Am. J. Path. **21**: 889, 1945.
15. Macklin, C. C.: The Transport of Air Along Sheaths of Pulmonic Blood Vessels From Alveoli to Mediastinum, Arch. Int. Med. **64**: 913, 1939.
16. Schwerma, H., and Ivy, A. C.: Safety of Modern Alternating Positive and Negative Pressure Resuscitators, J. A. M. A. **129**: 1256, 1945.

LABORATORY METHODS

AN APPARATUS FOR MEASURING THE RETAINED DOSE OF INHALED SUBSTANCES

JULIAN M. TOBIAS* AND RAYMOND E. WESTON†
EDGEWOOD ARSENAL, MD.

THE biologic effect of a given substance is usually assayed by determining that quantity per unit of body weight which produces specific changes under well-defined conditions. Such assay is not difficult for substances which may be injected or ingested without changes in physiologic activity, since the administered dose can be accurately measured. However, for inhaled substances the determination of dose is much more difficult.

To circumvent technical difficulties in actual dose measurement, inhaled substances have been assayed by exposing animals for a measured period of time to a known concentration of the test material. Since the work of Haber,[1] such exposures have been measured in terms of the lethal index or Ct (the product of the concentration of material times the minutes of exposure) in an effort to obtain some measure of dose. However, the concentration-time product is merely a measure of physical exposure and can never constitute an accurate estimate of the dose retained. The latter depends not only on the Ct but also on the percentage retention and the respiratory intake during exposure and is given by

$$D = \frac{VTC\alpha}{W}$$

where D = the retained dose in milligrams per kilogram, V = respiratory intake during exposure in liters per minute, T = exposure duration in minutes, C = concentration of material inhaled in milligrams per liter, α = percentage of inhaled material retained, and W = body weight in kilograms. Since both the percentage retention and the respiratory minute volume during exposure may vary widely when animals of the same or different species are exposed, animals exposed simultaneously to the same Ct may retain markedly different doses. Consequently, it is not surprising that extensive individual and species variations in response to different inhaled toxic materials have been observed when the Ct is the index of dose administered.

For the proper evaluation of the effect of inhaled substances the actual dose retained must be measured. Were this possible, several lines of investigation would be facilitated. For example, in the evaluation of aerosol therapy[2] the efficacy of a given inhaled dose of a therapeutic agent could be compared with

From the Physiology Section, Medical Division.
Received for publication, April 17, 1946.
*On leave from the Department of Physiology, University of Chicago.
†Captain, Medical Corps, Army of the United States.

that of a dose administered by one of the conventional routes. In addition, volatile industrial hazards could be assessed more accurately. Finally, in studies of the pulmonary blood-air barrier it would be possible to compare the amount retained in the lung with that transported across the semipermeable membrane.

Tobias, Patt, Swift, and Gerard[3]* developed a method for measuring the retained dose of an inhaled substance, which involved the use of the following: a dynamic chamber from which the animal withdraws air containing the test material at known concentrations, a valve system to direct the flow of air, an absorber to remove quantitatively any of the material which is expired, and a spirometer to record the volume of the expired air. The product of the chamber concentration times the volume inspired gives the quantity of the test substance removed from the chamber. The difference between this and the quantity in the absorber, as determined by chemical analysis, is the dose retained by the animal. Critical evaluation of their early experiments reveal that, although the method was correct in principle, there were several sources of error in the apparatus employed. Moreover, it was not applicable to unanesthetized animals without tracheal intubation.

As a result an apparatus has been developed which has the following characteristics:

1. All surfaces which come into contact with the test substance are made of glass or other chemically inert materials.

2. The resistance at ordinary respiratory air flows is low.

3. Condensation of expired moisture on surfaces of the valves is prevented, thus minimizing adsorption and surface hydrolysis.

4. Unanesthetized (or anesthetized) animals can be used with minimal discomfort.

DESCRIPTION OF APPARATUS

Specific dimensions given herein are not critical. The size of the apparatus should depend upon the species studied. The measurements given have been found satisfactory for the dog and the goat.

Valves.—Since the conventional valves contain some rubber, metal, or plastic materials which react with many chemical substances, valves consisting entirely of glass were developed. After preliminary trials with several designs, the present type, using standard tapered joints as valve housings, was selected because it combines simplicity of construction with low resistance to ordinary air flows (Fig. 1).

A circular glass disc (*A* in Fig. 1), 24 mm. in diameter and 1.5 mm. thick, is ground on one surface until an airtight seal is obtained, when the disc is seated on a similarly ground end of the male half of a 24/12 standard tapered, glass joint (*B* in Fig. 1). The grinding is accomplished in a few minutes by rubbing the surface of the disc and the valve seat on a piece of plate glass, using carborundum in water as the grinding agent. The cross sectional diameter of

*The present authors wish to acknowledge their indebtedness to the early collaboration of Dr. R. W. Gerard, Dr. H. M. Patt, and Miss M. N. Swift. Due to the exigencies of wartime research, these investigators were unable to participate in the development of the new apparatus, but their earlier assistance, planning as well as technical, was invaluable.

the airway should be large enough to pass the expected airflows with minimal resistance, but the total volume of the system should be as small as possible to diminish rebreathing by decreasing the dead air space.

As can be seen from Fig. 1, there is a circumferential dilation of the glass tubing for a short distance distal to the female half of the joint. Thus, the valve disc, when in position in the closed joint, can move in this dilated area during each respiratory cycle. When the disc is seated on the ground end of the male half of the joint, the valve is closed; when it is moved away from the end of the male half, the valve is open. The discs can shift sufficiently in this dilated space to allow passage of air with very little resistance (see paragraph on Resistance of Combined Valves and Absorber). The valves function most efficiently and rapidly with the long axes vertical and valve seats below, so that the discs are seated by gravity. Thus, the resistance which the valves offer is determined primarily by the weight of the discs.

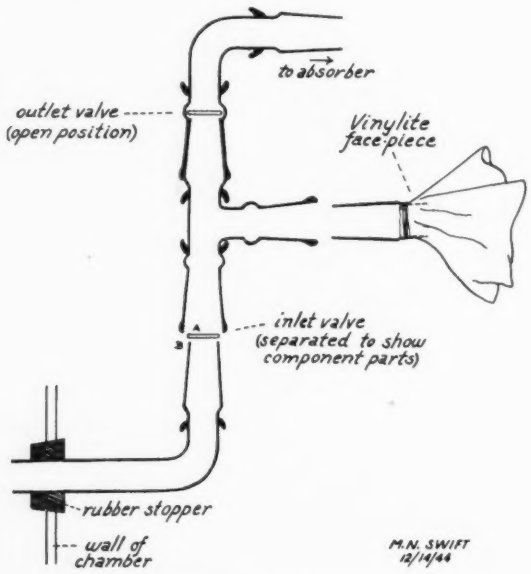

Fig. 1.—Valves and facepiece.

The entire valve assembly is wrapped with appropriate lengths of No. 24 gauge Nichrome wire insulated with asbestos tape. By regulating the current through the wire with a rheostat, the temperature of the glass is maintained slightly above body temperature to prevent condensation of water vapor from the expired air. Thus adsorption and hydrolysis on the glass surfaces are minimized. Sticking of the valves because of accumulated moisture is also avoided.

Absorber.—The absorber (Fig. 2) consists of a vertical, 48 mm. outer diameter glass cylinder, tapered to a 3 mm. stopcock below and to a ground glass stopper above. The inlet tube (20 mm. outer diameter) is connected to the expiratory valve by a ground glass joint and enters the absorber about 5 cm. above the stopcock. Then it bends sharply downward to terminate in a rounded blind end

which is perforated with a sufficient number of holes (about fifteen), 3 mm. in diameter, to permit free flow of expired gas into the absorber. The absorber is filled to a height of 23 cm. with 4 mm. solid glass beads which, when wet with a suitable absorbent solution, provide a large absorbing surface. However, since beads would plug the inlet holes, the lower 9 or 10 cm. of the absorber are filled with readily draining solid glass spirals. These are of such size and shape that they cannot obstruct the inlet openings. The outlet tube (20 mm. outer diameter) leaves the absorber above the level of the glass beads and leads to a recording spirometer.

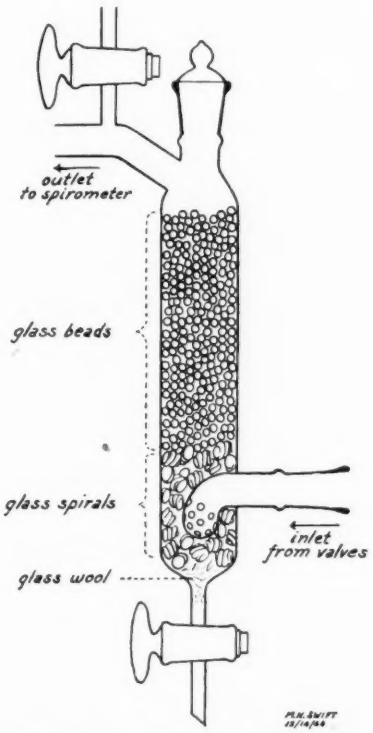

Fig. 2.—Low resistance absorber.

Immediately before use the inlet tube is closed with a glass stopper and the absorber filled with the best solvent for the substance being studied. Then it is shaken vigorously to remove any air bubbles trapped among the beads which might prevent some of the surfaces from becoming covered with solvent, and the solvent is drained from below. The stopper is removed and the inlet joint dried and attached to the valve outlet. After use the absorber is disconnected from the valves and the glass stopper replaced in the inlet. The beads, spirals, and inlet tube are washed thoroughly by repeated rinsings introduced from above. The several washings are drained through the bottom stopcock into a volumetric flask, and an appropriate aliquot is analyzed. The absorber need not be cleaned additionally for the next sampling. All that is required is to dry the inlet tube and to repeat the steps outlined.

Facepiece (Figs. 1 and 3).—The unanesthetized animal may be attached to the apparatus by means of a roughly cone-shaped, Vinylite facepiece which is sealed to the male half of a ground glass joint. The animal's mouth is held closed with Ace elastic bandage, care being taken not to occlude or to compress the nostrils, and the facepiece is slipped over the taped snout until the inner end of the glass joint nearly touches the animal's nose. More elastic bandage is wrapped around the facepiece and snout, thus diminishing the dead space. By means of the joint the animal is attached to the valve system (Fig. 3). Thus, the mouth is held closed to prevent leakage of saliva into the system, and an air-tight connection between the animal and the apparatus is achieved. The procedure apparently causes little discomfort. Some untrained animals require no restraint and stand quietly breathing into the apparatus.

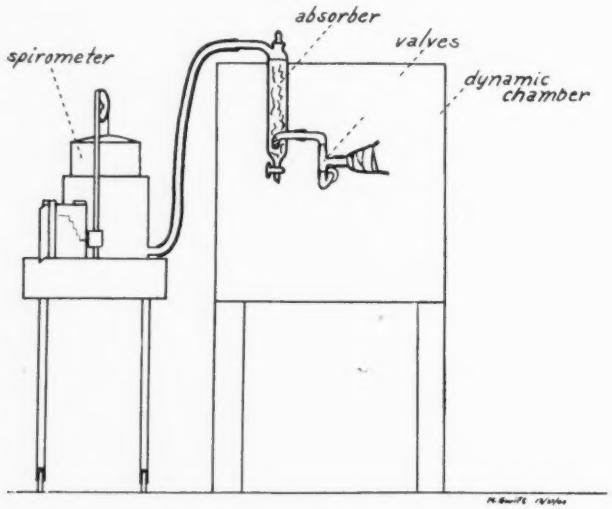

Fig. 3.—Assembled apparatus.

With care the facepiece can be applied on dogs and goats without leakage at the usual air flows. A modification of the facepiece which permits no leakage has been developed for use in monkeys.[5] However, as yet, no chemically inert, leak-proof facepiece has been found for use on rabbits.

In experiments in which severe hyperpnea may occur, the increased pressure of expiration may cause significant leakage. Therefore, it has been found advisable to test for leakage before experiments.[4] In this test the entire apparatus is assembled and the animal attached as usual. However, the inlet valve instead of being connected to the experimental atmosphere is joined to a calobrated spirometer. The animal then inspires air from one spirometer and expires into a second. Any leak would be indicated by a discrepancy between the volume changes in the two spirometers. If there is no leak, the inlet valve can be switched immediately to the experimental atmosphere and the experiment begun. When significant leakage has been noted, substitutes for the facepiece have been employed successfully with the valves and absorber. The upper

respiratory tract can be short-circuited by tracheal or nasal intubation,[4] both under general anesthesia, or by tracheotomy under local anesthesia.[5]

TESTS OF APPARATUS

Valve Efficiency.—To test the valves for leakage, the facepiece was replaced by a calibrated 50 c.c. syringe joined to the system by a rubber stopper. By repeated strokes of the syringe plunger measured amounts of air were drawn through the inlet valve and expelled through the outlet valve into a spirometer previously calibrated with the same syringe. With no leakage there should be 100 per cent recovery of the air pumped through the valves. Repeated tests (Table I) revealed essentially zero leakage.

TABLE I. TESTS FOR VALVE LEAKAGE

NUMBER OF SYRINGE STROKES	TOTAL VOLUME PUMPED BY SYRINGE (C.C.)	VOLUME RECOVERED IN SPIROMETER (C.C.)	DIFFERENCE	
			C.C.	%
60	3000	3000	0	0
60	3000	2980	20	0.7
60	3000	3000	0	0
60	3000	3000	0	0
60	3000	3000	0	0
59	2950	2950	0	0
58	2900	2900	0	0
40	2000	2000	0	0
40	2000	1990	10	0.5
40	2000	2000	0	0
20	1000	1000	0	0
20	1000	1000	0	0
20	1000	1000	0	0

Absorber Efficiency.—Several types of experiments were performed to establish the efficiency of the absorber. The test substance was a gas containing chloride. Chloride ion in the absorber (and in the standard Vigreaux bubblers used for comparison) was titrated potentiometrically in a modified Pincof cell.[6]

Three one-liter samples of air, containing from 7.3 to 10.8 $\times 10^{-5}$ mols of Cl per liter, were drawn, first through the absorber (beads wetted with 5 per cent NaOH) and then through a standard Vigreaux bubbler in series with it. With flow rates of from 750 to 1,500 c.c. per minute no Cl was found in the second bubbler in six trials. Therefore, the bead absorber must have removed all the test gas from the air passing through it.

Again by steady suction, nearly simultaneous, three liter samples of Cl laden air were drawn from a dynamic chamber, one through the valves and bead absorber and another through a standard bubbler. In Table II recovery by the bead absorber and bubbler are compared. The percentage difference be-

TABLE II. CHLORIDE RECOVERY BY BEAD ABSORBER AND STANDARD BUBBLER (STEADY GAS FLOW)

CL RECOVERY		DIFFERENCE	
BEAD ABSORBER (MOLS $\times 10^{-5}$ PER LITER)	STANDARD BUBBLER (MOLS $\times 10^{-5}$ PER LITER)	MOLS $\times 10^{-5}$	%
17.75	17.70	0.05	+0.3
17.34	17.53	0.19	−1.0
17.70	17.44	0.26	+1.4

tween the two sets of values is small enough to have resulted from a summation of possible errors in titrations or volume measurements.

To approximate more closely the conditions of an actual experiment, the valves, absorber, and spirometer were set up in series. The chloride containing gas was pumped through the apparatus by a 50 c.c. syringe (simulating a breathing animal), from a chamber the gas concentration of which was simultaneously and independently analyzed. The data in Table III further establish the efficiency of the bead absorber. Chloride recovered from the absorber is seen to have agreed very well with the value predicted from standard chamber analysis.

TABLE III. CHAMBER CHLORIDE CONCENTRATION DETERMINED BY STANDARD BUBBLER AND BEAD ABSORBER WITH VALVES INTERPOSED (INTERMITTENT GAS FLOW)

VOLUME OF GAS PUMPED (C.C.)	CL CONCENTRATION IN CHAMBER (MOLS × 10^{-5} PER LITER)	BEAD ABSORBER CL (MOLS × 10^{-5} PER LITER)		DIFFERENCE	
		EXPECTED	MEASURED	MOLS × 10^{-5} PER LITER	%
3,000	5.67	17.01	16.98	0.03	−0.2
3,060	5.51	16.85	16.88	0.03	+0.2
3,050	5.51	16.80	16.83	0.03	+0.2
2,980	5.21	15.53	15.61	0.08	+0.5
2,980	5.13	15.29	15.35	0.06	+0.4
3,000	5.14	15.42	15.66	0.24	+1.5

Resistance of Combined Valves and Absorber.—To measure resistance of the apparatus at normal air flows, a calibrated membrane manometer was connected between the inspiratory valve and the facepiece. Thus, in addition to the spirometer measurement of tidal air and respiratory rate, a record could be obtained of pressure changes during respiration. After the temperature of the heating coil was adjusted and the beads in the absorber were moistened, dogs were permitted to breathe into the apparatus through the usual facepiece.

TABLE IV. RESISTANCE OF COMBINED VALVES AND ABSORBER

RESPIRATION PER MINUTE	TIDAL AIR (C.C.)		INSPIRATORY PRESSURE (MM. H_2O)		EXPIRATORY PRESSURE (MM. H_2O)	
	MIN.	MAX.	MIN.	MAX.	MIN.	MAX.
12	148	390	4.4	4.4	9.0	12.8
12	117	234	4.4	4.4	6.7	12.2
8	179	312	4.0	4.4	7.7	13.4
10	273	468	4.5	12.2	12.2	17.5
10	214	308	4.2	4.4	10.2	13.2

From the data in Table IV it is clear that both the inspiratory and expiratory pressures at different respiratory rates and tidal air flows are no greater than the normal intratracheal pressures. Therefore, the entire system, facepiece, valves, wet absorber, and spirometer, offers but slight resistance to normal respiration.

DISCUSSION

The apparatus described for determining retained doses of inhaled substances fulfills the essential specifications. All parts coming into contact with

respired mixtures are constructed of glass or other chemically inert materials. No condensation of exhaled water vapor can occur within its channels. Since the valve flaps are light in weight, since the diameter of the tubing is relatively large, and since the absorber utilizes a large wet surface rather than a liquid column, the resistance offered by the apparatus at normal respiratory flows is low. Unanesthetized animals can be used with a minimum of discomfort.

A factor to be considered is the quantitative relation between the volumes of inspired and expired air, because the volume breathed during exposure is measured as expired air. Actual measurements done in connection with leakage tests (see paragraph on Valve Efficiency) have detected no significant difference. It is, however, obvious that when an alkaline solution is used in the absorber CO_2 will be removed from the expired air. Consequently, since the animal consumes oxygen, the expired volume will be smaller than the inspired. The discrepancy would be about 4 to 6 per cent and will tend to make the retained dose too low. If greater accuracy is required, the discrepancy can be corrected approximately by calculation or accurately by determining the oxygen content of the expired air.

The valves, it should be recalled, constitute two outlets of a glass tube with a third outlet where the facepiece attaches. Thus, the volume delimited by the inspiratory valve disc below, the expiratory valve disc above, and the facepiece constitutes a "dead space" through which the animal must breathe. Consequently, at the end of each expiration the concentration of test substances in the dead space will be somewhat less than that in the chamber, the extent of the dilution depending on the concentration of test material in the expired air and the relationship between the volume of the dead space and the animal's tidal air. Conversely, during inspiration, as the experimental mixture is drawn from the chamber, the concentration in the dead space is increased by an increment which depends also on the dead space-tidal air relationship. At the end of inspiration some of this more concentrated mixture is expired into the absorber without coming into contact with the animal. As a result analysis of the absorber contents will yield a somewhat excessive estimate of the amount of material to which the animal was exposed, and if not corrected, the apparent percentage retention will be too low. On the other hand the concentration to which the animal is exposed will be less than that in the chamber due to the dilution in the dead space by the expired air. The detailed mathematic treatment and derivation of a correction factor for calculating the true percentage retention and the actual exposure concentration appear elsewhere.[5] It is wise to reduce the dead space as much as possible by using short standard tapered joints. However, it should be emphasized that the existence of the dead space has no influence on the determination of the retained dose.

CONCLUSIONS

1. An apparatus has been constructed which permits accurate measurement of the actual doses of certain inhaled substances retained by unanesthetized animals.

2. The percentage of inhaled material retained may be calculated readily.

3. There is very little resistance to normal respiratory air flows.

4. The essential parts of the apparatus are entirely of glass and easily obtainable.

The authors are grateful for technical assistance by Mrs. Harriet Kriete, Mr. Thomas Keithley, and Dr. Carl B. Marquand.

REFERENCES

1. Haber, R.: Fünf Vortrage aus den Jahren 1920-1923, No. 3. Die Chemie in Kriege, No. 5. Zur Geschichte des Gaskampfes, Berlin, 1924, Julius Springer.
2. Bryson, V., Sansome, E., and Laskin, S.: Aerosolization of Penicillin Solutions, Science **100**: 33-35, 1944.
3. Tobias, J. M., Patt, H. M., Swift, M. N., and Gerard, R. W.: Unpublished data.
4. Blivaiss, B., Byerrum, R., Rotariu, G., Tobias, J. M., and van Hoesen, D.: To be published.
5. Weston, R. E., and Karel, L.: In press.
6. Epstein, J., and Sober, H. A.: Personal communication.

THE RELATIVE EFFICIENCY OF WATER CENTRIFUGAL SEDIMENTATION AND OTHER METHODS OF STOOL EXAMINATION FOR DIAGNOSIS OF SCHISTOSOMIASIS JAPONICA

SECOND LIEUTENANT BAHIJ J. BAROODY, SANITARY CORPS, AND
MAJOR HARRY MOST, MEDICAL CORPS, ARMY OF THE UNITED STATES

THE demonstration of the eggs of *Schistosoma japonicum* in American personnel under clinical observation for suspected schistosomiasis frequently entails numerous stool examinations. Patients with light or subclinical infections or those following inadequate treatment may have repeatedly negative stools by the ordinary methods of examination. Initial treatment or retreatment is usually not given unless the stools are positive. It is, therefore, desirable to employ methods with the highest degree of efficiency in detecting eggs for diagnosis and subsequently in the evaluation of therapy. During the past year we have examined more than 25,000 stool specimens from 800 patients with suspected or proved schistosomiasis japonica. In the course of these examinations the relative efficiency of various stool techniques was studied. The purpose of this paper is to present a simple rapid method of stool examination which we have found to be more efficient in detecting eggs of *S. japonicum* than other routine or special methods currently employed.

MATERIAL AND METHODS

Each initial stool specimen from all patients suspected of having schistosomiasis was examined by direct smear, zinc sulfate centrifugal flotation, brine flotation, and the modified centrifugal water sedimentation method described herein. All subsequent stool specimens were examined by direct smear, special attention being given to select a portion containing blood or mucus if present, and by centrifugal water sedimentation. A selected number of positive specimens was submitted to comparative study by the acid-ether, zinc sulfate, direct smear, and centrifugal water sedimentation methods of stool examination as well as several modifications of these techniques.

Faust and Meleney,[1] in 1924, described a water sedimentation method of stool examination for the demonstration of eggs and miracidia of *S. japonicum*. This method requires the use of the whole fecal specimen and repeated washing and gravity sedimentation in glass containers of from 1,000 to 2,000 c.c. capacity. The major value of this method apparently lies in the large amount of feces employed, but it is not practical for routine use in large numbers of examinations because of the size of the vessels required and the time consumed in its use (from one to two hours or more before final sediment is ready for examination). However, it may be of value in an individual case with extremely few eggs if stools are repeatedly negative by other methods in which relatively small amounts of feces are used.

Received for publication, April 26, 1946.

Modified Water Centrifugal Sedimentation Method.—

1. Place from 10 to 15 Gm. of the feces in a 125 c.c. Erlenmeyer flask containing about 100 c.c. of lukewarm tap water. Insert a No. 4 rubber stopper and shake vigorously for one or two minutes, or until the particles are in fine suspension.

2. Strain the emulsified feces through two layers of wet gauze into a 50 c.c. centrifuge tube with teated bottom (tube dimensions: height, 4¾ inches; diameter, 1 inch).

3. Centrifuge at 1,500 r.p.m. for thirty seconds (brake of centrifuge applied gradually).

4. The supernatant fluid is decanted and tap water of about 40° C. is added to the 50 c.c. mark. Insert a rubber stopper and shake several times until the sediment is finely dispersed. The warm water causes a scum to rise to the surface. This is desirable.

5. Repeat procedures 3 and 4 until the supernatant fluid is clear; three washings and three centrifugations are usually sufficient.

6. After the last washing and centrifugation the clear supernatant fluid is poured off and the packed sediment remains. If the finger is gently tapped against the side of the tube, the packed sediment is dispersed and settles to the bottom of the tube by gravity within a minute.

7. With a rubber-bulbed pipette place 4 drops of mixed sediment on a glass slide and cover with a large cover slip size 22 by 40 mm. Make at least two slides.

8. Examine under low-power objective of the microscope for eggs of schistosoma. *S. japonicum* eggs are oval, 70 to 100 by 50 to 60 μ, may or may not have a rudimentary spine, and in blue-white light appear greenish orange. *Schistosoma mansoni* eggs are elongated, 114 to 175 by 45 to 68 μ, and have large lateral spines. In both species, if the ova are viable, the miracidia will exhibit movements within the eggshells, and the activity of the flame cells may be seen under higher magnification.

9. If the slides are negative for schistosoma ova, add about 10 drops of water to the remaining sediment in the tube, leave overnight in a warm place, and examine the following day for miracidia. The entire contents of the tube can be placed on a large slide and examined under a dissecting microscope. This phase of the examination requires less than two minutes.

10. Excluding procedure 9, not more than five minutes are required for the examination of each slide, and not more than from fifteen to twenty minutes are required from procedures 1 through 8.

Summary of the Method:

1. A large sample of the feces is used; this is especially valuable in light infections.

2. Viability of the ova is determined.

3. Rapidity of method—report can be rendered within fifteen minutes.

4. A hatching test is a part of the method.

5. Other ova, cysts of protozoa, and larvae of helminths may also be found and identified. (For protozoan cysts add a drop of Lugol's or D'Antoni's iodine to slide.)

6. Preserved concentrated specimens can be made quickly by adding 10 per cent formalin to the sediment in the tube at the end of the fresh examination.

7. No chemical reagents are required; minimum standard laboratory equipment is used.

Comparative Results of Stool Examinations by Water Centrifugal Sedimentation and Other Methods.—

1. *Direct Smear:* 1,026 consecutive fecal specimens (171 patients) not containing any grossly visible blood or mucus were examined simultaneously by direct smear and centrifugal sedimentation. These patients all had received treatment previously and were under observation for the evaluation of success or failure of various forms of therapy. Patients who were found to have positive results were examined daily until another positive stool was found. Results in ten patients were found positive by direct examination of fecal material free of grossly visible blood or mucus. Results in these were all positive in water centrifugal flotation. In addition, results in thirty-nine patients were found positive by the latter method making a total of forty-nine positive cases detected by centrifugal water sedimentation or a relative efficiency of 4.9:1 for this method compared to direct smear examination. This particular run is considered a good test of both methods since all patients had been treated previously, and those with positive results were passing only a few eggs. In view of the fact that all patients with schistosomiasis in Army personnel diagnosed overseas or in this country will have received one or more courses of treatment, it may be expected that the majority of such patients still having active infections will escape detection by direct smear examination if no mucus or blood is present in the feces. The conditions in this test run will simulate those faced by physicians and laboratories in civil or veteran's facility practice.

2. *Examination of Mucus or Bloodstreaked Mucus by Direct Smear:* All stool specimens were examined carefully by inspection of the surface and crevices for the presence of areas of bloody or mucoid material. Such material was placed on several slides and examined microscopically. Of fifty consecutive specimens from different patients whose stools contained bloody mucus, forty-five (or 90 per cent) were positive for eggs of *S. japonicum* on direct smear examination of this material. Usually a large cluster of eggs was readily found under low-power magnification within a minute. Of the forty-five specimens which were positive on direct examination, forty-two were also positive by water centrifugal sedimentation performed on a portion of the same fecal specimen but from an area not containing visible mucus or blood. In addition, one of the five bloody mucus specimens which were negative by direct smear was positive by water centrifugal sedimentation. Actually, for diagnosis in this group of fifty patients, it would have been necessary to run by sedimentation only the five specimens which were negative on direct examination of bloody mucus. The total positive results in the group would

then be forty-six (or 92 per cent), of which direct smear alone had detected 97.8 per cent of the positive results. In this group of fifty 86 per cent of the total patients were detected by centrifugation alone.

Consideration of the findings discussed in the preceding two paragraphs have these practical implications. First, careful gross examination of stool specimens for the presence of bloody mucus is very important, because in this group of patients if the latter was found we could expect to find eggs of *S. japonicum* readily and quickly by direct examination of such material in about 90 per cent of the specimens. No further examination then is necessary. Second, direct smear examination of nonbloody mucous fecal material in the majority of patients particularly in those with light infections or after one or more courses of treatment, will yield relatively few positive results and only rare positives will occur which are negative by the water centrifugal sedimentation method. In our experience the latter method has confirmed all routine direct smear positives obtained from fecal specimens free of grossly visible bloody mucus. A reasonable technique therefore in a stool examination for suspected schistosomiasis is careful inspection of the specimen for bloody mucus, direct examination of the latter if present, and water centrifugal sedimentation if negative or not present.

3. *Zinc Sulfate Centrifugal Flotation:*[2] In the course of these studies approximately 600 patients with schistosomiasis had at least one examination by the foregoing method. The diagnosis of schistosomiasis was confirmed by the demonstration of eggs of *S. japonicum* at this hospital in 226 patients, but in *no* case was the diagnosis made by zinc sulfate. Ten specimens containing a large number of *S. japonicum* eggs were studied by the zinc sulfate method. In two specimens wrinkled masses of eggshells were found by zinc sulfate, but proper identification would have been impossible. If the speed of centrifugation is reduced below 1,000 r.p.m., distortion is less marked, but the eggs are still not suitable for routine identification. If the whole sediment obtained by water sedimentation is treated by the zinc sulfate method but centrifuged at less than 1,000 r.p.m., a large number of eggs will be brought to the surface. These are also poorly preserved, and this modification would have no merit since it would entail carrying out the procedure of two methods, the first of which alone gives a more satisfactory result. The standard zinc sulfate method routinely employed in patients suspected of having schistosomiasis is valuable in detecting protozoan cysts and larvae and eggs of some helminths but is of no value in the diagnosis of *S. japonicum* infections.

4. *Brine Flotation:* No patients with schistosomiasis japonica were discovered by this method which was applied at least once in all patients.

5. *Acid Ether:* Fifty specimens from different patients found to have positive results by water centrifugal sedimentation were examined by the acid-ether techniques of De Rivas[3] and Weller and Dammin.[4] The use of 40 per cent hydrochloric acid was superior to 5 per cent acetic acid, but both methods of acid ether yielded only sixteen positive results or an efficiency of only 0.32 compared to water centrifugal sedimentation (1.00). Ten specimens positive by

water centrifugal sedimentation but negative by acid ether were examined again by the acid-ether method with the addition of small amounts of quartermaster laundry detergent. Only one specimen became positive as a result of this modification, and it was not deemed worth while to explore this further. Acid ether alone was superior to direct smear examination of specimens without visible bloody mucus, but either method or both combined were far inferior to water centrifugal sedimentation alone and did not increase the yield of positives if performed in conjunction with the latter. Acid ether is not recommended as the procedure of choice for the diagnosis of schistosomiasis japonica infections.

6. *Comparison of All Methods in a Series of Fifty Consecutive Stools Positive for Eggs of S. japonicum:* The findings summarized in Table I bear out the data presented and discussed in the preceding paragraphs. One notes that in this series zinc sulfate and brine flotation failed to detect a single infection.

Examination by direct smear of bloody mucus present in eight specimens was positive in seven, and the remaining specimen was positive by water centrifugal sedimentation. The latter method alone would have missed three specimens positive by direct smear. Examination of feces from areas not containing grossly visible bloody mucus by direct smear detected nine (18 per cent) positives, all of which were also positive by water centrifugal sedimentation and four of which were positive by examination of mucus from the same specimen. Direct smear examination of fecal material with and without bloody material thus detected twelve (or 24 per cent) of the total patients with positive results in this series.

The acid-ether method permitted detection of sixteen positive results (32 per cent), all of which were also positive by water centrifugal sedimentation. Acid ether detected seven positives not discovered by direct smear of fecal specimens free of grossly visible bloody mucus. The one specimen of bloody mucus which was negative by direct smear was also negative by acid ether, and three positive bloody mucus specimens were negative by acid ether. Acid ether alone, therefore, would have discovered 32 per cent of the positives, and, if combined with direct smear of specimens with or without bloody mucus, the acid-ether method would have detected nineteen (or 38 per cent) of the total positives.

Water centrifugal sedimentation detected forty-seven (or 94 per cent) of the total positive results in this group of fifty patients; the remaining three having been found to have positive results by direct smear of blood mucus and negative by water centrifugal sedimentation.

Consideration of these data indicates that the most practical and efficient method of detection of eggs of *S. japonicum* in stool specimens consists of (1) careful visual examination of the entire gross specimen for the presence of mucus or bloody mucus and, if present, the latter is examined by direct smear and (2) water centrifugal sedimentation examination if mucus or bloody mucus is absent or negative by direct smear examination if present.

Number of Stool Examinations for Diagnosis and Discovery of Treatment Failures.—In the presence of active infection with *S. japonicum* several factors

TABLE I. RESULTS OF STOOL EXAMINATIONS FOR EGGS OF S. JAPONICUM BY VARIOUS METHODS IN FIFTY PROVED CASES

CASE	SMEAR BLOODY MUCUS	DIRECT SMEAR	ACID ETHER	CENTRIFUGAL SEDIMENTATION	ZINC SULFATE	BRINE FLOTATION
1		0	0	X	0	0
2	P X	X	X	X	0	0
3		X	X	X	0	0
4		0	0	X	0	0
5	P X	0	0	0	0	0
6		0	0	X	0	0
7		0	X	X	0	0
8		0	0	X	0	0
9	P X	X	X	X	0	0
10		0	X	X	0	0
11		0	0	X	0	0
12		0	0	X	0	0
13		0	X	X	0	0
14		0	0	X	0	0
15		0	X	X	0	0
16	P X	0	0	0	0	0
17		0	0	X	0	0
18	P O	0	0	X	0	0
19		0	0	X	0	0
20		0	0	X	0	0
21		0	0	X	0	0
22		0	0	X	0	0
23	P X	X	X	X	0	0
24		0	0	X	0	0
25		0	0	X	0	0
26	P X	0	0	0	0	0
27		X	X	X	0	0
28		0	0	X	0	0
29		X	X	X	0	0
30		X	X	X	0	0
31		0	0	X	0	0
32		0	0	X	0	0
33	P X	X	X	X	0	0
34		0	0	X	0	0
35		0	X	X	0	0
36		0	0	X	0	0
37		0	0	X	0	0
38		0	0	X	0	0
39		0	0	X	0	0
40		0	X	X	0	0
41		0	0	X	0	0
42		0	0	X	0	0
43		0	X	X	0	0
44		0	0	X	0	0
45		0	0	X	0	0
46		0	0	X	0	0
47		X	X	X	0	0
48		0	0	X	0	0
49		0	0	X	0	0
50		0	0	X	0	0
Total		9	16	47	0	0

P, present; X, positive; 0, negative.

other than the efficiency of the technique of stool examination will have an influence on whether or not eggs are found in the feces. These are the stage of the disease, the degree and extent of intestinal involvement, whether specific treatment has been given, and, if so, the drug and total amount, the time interval following completion of treatment when stool examinations are be-

gun, and the total number of stools examined. These factors will be discussed briefly.

The acute stage of the disease will not ordinarily be seen in this country. However, the number of stool examinations necessary to establish the diagnosis during the acute stage of the disease experienced overseas is of interest. Of fifty patients with positive stools overseas subsequently found to have positive stools at this hospital, results in twenty-two were found positive overseas on the first examination, seventeen in from two to five examinations, ten in from six to ten, one was positive in from eleven to twenty, and one in more than twenty stool examinations. The average number of stool examinations necessary to demonstrate eggs of *S. japonicum* during the acute stage of the disease overseas in this series of patients who were examined within from two to four months after first possible exposure was 3.6. The method of stool examination at overseas hospitals was not specified but probably consisted of direct smear and some form of water sedimentation. Stool examinations in these same patients were all found positive here after prior treatment. In nine patients results were positive in the first five examinations, ten in from six to ten, eleven in from eleven to fifteen, six in from sixteen to twenty, seven in from twenty-one to twenty-five, and seven in from twenty-six to thirty-five stool examinations. The average number of stools examined before eggs were found was fifteen. It is apparent then that eggs are found more readily and with fewer stool examinations during the acute stage of the disease than later after treatment has been given.

In order to determine whether eggs once present appear regularly thereafter, stools from patients found positive were examined daily until the next positive result was found. Of fifty such patients, results in thirty-seven were positive on two consecutive days, five again on the third consecutive examination, two on the fourth, two on the eighth, and one each on the fourteenth, nineteenth, twentieth, and twenty-second consecutive examinations. This indicates the irregularity with which eggs of *S. japonicum* may appear in the stools, since from one to twenty-two days or more may elapse between two positives and emphasizes the necessity for repeated examinations before the diagnosis can be excluded reasonably.

Success in discovering treatment failures is governed largely by consideration of the type and amount of treatment, the total number of stools examined, and when, after treatment, such examinations are begun and how long continued. Stool examinations during the first three or four weeks immediately after treatment are of no significance since eggs already laid prior to treatment may require this length of time to leave the body and do not indicate treatment failure.[5] As a rule treatment failures after fuadin are discovered at shorter intervals (from five to seven weeks) than after tartar emetic (from eight to twelve weeks following completion of treatment). Fifty consecutive treatment failures at Moore General Hospital required an average of eight stool examinations (ranging from one to twenty-nine) during an interval of sixty days beginning thirty days after completion of treatment before eggs were discovered. It is therefore suggested that stool exam-

inations be started thirty days after completion of treatment and such examinations be continued (three specimens per week) for the next eight to ten weeks. Since the majority of patients with schistosomiasis japonica will require one or more courses of treatment, consideration of these factors is important in discovering treatment failures. Treatment failures can only be discovered by repeated stool examinations during a relatively long period of time. In evaluating suspected schistosomiasis in patients with a definite history of exposure in an endemic area and suggestive clinical findings, from twenty to thirty stool examinations in from four to six weeks may be required to establish or exclude the presence of eggs in the stools. Persistently negative stools in some patients do not preclude the possibility of active infection.

Parasites Other Than S. japonicum Discovered by Water Centrifugal Sedimentation.—In the course of numerous examinations by the previously mentioned method of stool specimens from patients with suspected or proved schistosomiasis, other parasites were frequently discovered. In 100 patients found to have *S. japonicum* eggs in the stool as a result of one or more examinations by water centrifugal sedimentation, 4 per cent of the patients were found to have cysts of *Endamoeba histolytica* in the sediment, 18 per cent, 20 per cent, and 4 per cent had eggs of hookworm, *Trichuris trichiuria* and *Ascaris lumbricoides*, respectively, and 2 per cent had larvae of *Strongyloides stercoralis*. A single zinc sulfate examination in each of the same patients showed 4 per cent, 9 per cent, 4 per cent, 1 per cent, and 2 per cent to have these parasites in the order given. A single brine flotation in these patients detected 13 per cent to have hookworm infections, and six and 2 per cent had Trichuris and Ascaris, respectively.

No comparison of the relative efficiency of these methods in detecting protozoa or helminths is intended. Each patient with stools positive for *S. japonicum* had on the average fifteen water sedimentation examinations before the diagnosis was established and at least thirty additional examinations after treatment, whereas zinc sulfate and brine flotation were done only once except to evaluate treatment for hookworm or amebiasis. However, it is important to note that in the course of many water centrifugal sedimentation examinations for the diagnosis or evaluation of therapy in schistosomiasis other pathogenic protozoa or helminths may be found, which may or may not be found also in a single zinc sulfate, brine flotation, or direct smear examination.

SUMMARY AND CONCLUSIONS

1. Water centrifugal sedimentation is a simple rapid method of stool examination for detecting eggs of *S. japonicum* in the stools of infected patients.

2. This method requires no chemical reagents, and only a minimum amount of standard laboratory equipment is necessary.

3. In our experience it was more efficient than direct smear examination of stool material free of grossly visible bloody mucus or examination by acid-ether, zinc sulfate, or brine flotation. Viability of eggs found after treatment can be determined.

4. Direct smear examination of bloody mucus present in some stools revealed a high percentage of positives for *S. japonicum* in such material. Occasionally trophozoites of *E. histolytica* also were found in this material.

5. Water centrifugal sedimentation done repeatedly in the course of the diagnosis of suspected schistosomiasis, or for evaluation of specific therapy, will frequently detect the presence of other protozoa and helminths in addition to *S. japonicum*.

6. In our experience the stool method of choice in the study of patients with schistosomiasis japonica is as follows:

(a) Careful visual examination of the gross stool for the presence of bloody mucous material. Direct smear examination of this material if present.

(b) Water centrifugal sedimentation of all specimens not containing grossly visible bloody mucus and specimens negative by direct smear of bloody mucus if present.

(c) Routine zinc sulfate and brine flotation for the detection of protozoa and helminths other than *S. japonicum* which may or may not be found by water centrifugal sedimentation and which may be present in a high per cent of patients under observation for schistosomiasis japonica.

7. From twenty to thirty stool examinations during a period of from four to six weeks were required to detect light asymptomatic infections in a group of patients exposed on Leyte. Eggs had not been detected previously in any of these individuals, and none had received treatment.

8. Following treatment stool examinations should be begun thirty days after completion of treatment and continued for from eight to ten weeks (three stools weekly). Most treatment failures are discovered from five to twelve weeks after completion of therapy with fuadin or tartar emetic.

REFERENCES

1. Faust, E. C., and Meleney, Henry E.: Studies on Schistosomiasis Japonica, Am. J. Hyg., Monographic Series No. 3, p. 218, 1924.
2. Faust, E. C., D'Antoni, J. S., Odom, V., Miller, M. J., Peres, C., Sawitz, W., Thomen, L. F., Tobie, J., and Walker, J. H.: A Critical Study of Clinical Laboratory Technics for the Diagnosis of Protozoan Cysts and Helminth Eggs in Feces. I. Preliminary Communication, Am. J. Trop. Med. **18**: 169-183, 1938.
Faust, E. C., Sawitz, W., Tobie, J., Odom, V., Peres, C., and Lincicome, D. R.: Comparative Efficiency of Various Technics for the Diagnosis of Protozoa and Helminths in Feces, J. Parasitol. **25**: 241-262, 1939.
3. De Rivas, D.: An Efficient and Rapid Method of Concentration for the Detection of Ova and Cysts of Intestinal Parasites, Am. J. Trop. Med. **8**: 63-72, 1928.
4. Weller, T. H., and Dammin, G. J.: The Acid Ether Centrifugation and the Zinc Sulfate Flotation Techniques as Methods for the Recovery of the Eggs of Schistosoma Mansoni, Am. J. Trop. Med. **25**: 367-374, 1945.
5. Vogel, H.: Über Entwicklung, Lebensdauer und Tod der Eier von Bilharzia japonica im Wirtsgewebe, Deutsche tropenmed. Ztschr. **46**: 57 and 81, 1942.

A PRESUMPTIVE MEDIUM FOR DIFFERENTIATING PARACOLON FROM SALMONELLA CULTURES

MARTHA L. CHILTON, B.A., AND MACDONALD FULTON, PH.D.
GALVESTON, TEXAS

WITH the increasing attention that is being given to the bacteriologic characteristics and pathogenic significance of the paracolon bacilli, the need has arisen for prompt and effective tests by which they may be recognized. Essentially, the paracolon group is characterized by the single property of fermenting lactose slowly. This is not a satisfactory characteristic for use in diagnostic laboratory work because, as Monteverde[1] and others have well shown, it is very slow to appear, irregular even with the same pure culture, and in general it is an unpredictable reaction. The advantage of the prompt reporting of identifications, if the physician is to be guided in treatment by knowledge of the type of bacterial infection existing in the patient, makes less time-consuming tests very necessary.

Since Russell,[2] in 1911, advocated the first double sugar agar medium for the differentiation of gram-negative enteric bacilli on the basis of lactose and glucose fermentation, many workers have proposed similar one-tube test media. Among the more recent media are Hajna's,[3] Sulkin and Willett's,[4] Felsenfeld and Young's,[5] Krumwiede and Köhn's,[6] Kligler's,[7] and Friewer and Shaughnessy's.[8] Felsenfeld proposed using his medium not as a screen test but to confirm the reactions of organisms suspected of being Salmonella or Shigella on the basis of a previously performed test on Friewer and Shaughnessy's medium. The successive use of two media, the second of which had to be observed for four days, did not accomplish the objective of rapid separation of the Salmonella and paracolon groups.

The problem of prompt recognition of paracolon cultures was approached by comparing the fermentation reactions of over 250 paracolon strains with the reactions of the strains of Salmonella in the standard collection of Salmonella type cultures. There were various tests in which paracolon cultures gave positive results while Salmonella were negative. By combining these test substances into a single medium, a presumptive test medium was secured in which a positive reaction eliminated the possibility that the test organism belonged to the Salmonella group. The new multiple sugar medium was intended for use as a preliminary test with which to distinguish paracolon cultures from Salmonella, replacing lactose broth which had proved too slow and uncertain. For convenience this medium was called AASS, from the initials of the carbohydrates used in it.

From the Department of Pediatrics, University of Texas School of Medicine.
Aided by a grant from Sharp & Dohme, Inc., Philadelphia, Pa.
Received for publication, May 17, 1946.

The composition of the AASS medium was: 0.5 per cent adonitol, 0.5 per cent aesculin, 0.5 per cent salicin, and 0.5 per cent sucrose dissolved in purple broth base.* Two indicators were employed: (a) bromeresol purple, 0.015 Gm. per liter as present in purple broth base, and (b) ferric ammonium citrate brown scales, 0.05 Gm. per liter. The carbohydrates dissolved in the broth were tubed in from 2 to 3 c.c. amounts and sterilized in the usual manner. The AASS broth was also tested using gas vials, and it gave the same reactions within the same limits of time as the medium without the vials. Hence, gas production could also be observed in this medium if desired.

None of the four carbohydrates was fermented by Salmonella, whereas one or more of them was usually fermented by the paracolon organisms. The medium became yellow if adonitol, salicin, or sucrose was fermented. If aesculin was fermented the medium blackened. In addition to the fermentation of the sugars, Kovacs' test for indole production was superimposed on these cultures, thus making it possible to detect many paracolon strains which were indole positive but did not ferment the carbohydrates. In this connection it may be pointed out that these indole tests were made on cultures which failed to ferment any of the four carbohydrates which were present. As a matter of interest it can be recorded that indole tests were also made on the cultures which showed positive fermentation reactions, with results that agreed with the known indole reaction of the culture obtained in all cases. Over 250 paracolon strains were tested in the AASS medium, and it was found to be about 80 per cent efficient in indicating that a culture belonged to the paracolon group. This result was obtained with cultures incubated for twenty-four hours at 37° C.

The paracolon group could be separated into three types on the basis of biochemical reactions in the AASS medium: (1) those which fermented one or more of the carbohydrates in twenty-four hours, (2) those which did not ferment any of the sugars but were indole positive in twenty-four hours, and (3) those which did not ferment the sugars and which were indole negative in twenty-four hours. Forty-four per cent of the 257 cultures tested fermented at least one of the sugars in twenty-four hours. Some of these tubes were yellow indicating that adonitol, salicin, or sucrose had been fermented, while others were black indicating fermentation of aesculin. Thirty-five per cent were fermentation negative and indole positive. The indole tests checked with the results obtained by Kovacs' test in the usual 1.0 per cent tryptone broth. Twenty-one per cent of the paracolon strains were negative in both tests. AASS cultures of this group were observed for seven days, and only eleven of the strains were slow fermenters of the mixture of carbohydrates. Among this group of fifty-five cultures, twenty-two gave evidence of ability to ferment lactose when grown for twenty-four hours at 37° C. on a heavily inoculated slant of 10 per cent lactose in purple agar base.* This comparatively aerobic method of testing for ability to ferment lactose was found to be superior in many instances to any other broth semisolid or solid stab medium which was tested.

*Difco Laboratories, Inc., Detroit, Mich.

The AASS medium can be used in accordance with the following suggested routine: Possible paracolon cultures are inoculated into the AASS medium and onto slants of 10 per cent lactose agar containing bromcresol purple indicator. Incubate for twenty-four hours at 37° C. Positive reactions in AASS will be considered to be acidification, blackening, or a positive Kovacs' indole test. The 10 per cent lactose slant will be read positive when (a) the indicator in the agar is changed or (b) when daughter colonies showing the acid color of the indicator are observed arising on the growth on the slant. The identification of the paracolon group is indicated when either or both of these two tests is positive in twenty-four hours.

Proteus cultures may give a positive AASS test, as may Shigella. The latter can be excluded by the slide agglutination test as developed by Weil and associates.[9] The recognition of Proteus cultures as a group is best made by the test for hydrolysis of urea as proposed by Rustigian and Stuart.[10] Further investigations are needed in order to devise means for the prompt classification of the group of strains which were negative in AASS and on the 10 per cent lactose slant. At present the complete study of such strains is the only known way to evaluate them correctly, and this study is both cumbersome and time consuming.

AASS medium has the psychologic advantage of providing a positive reaction rather than the negative test results which all too often have been the sole basis for the classification of cultures in the paracolon group. The AASS medium provides a rapid means of recognizing paracolon cultures and hastens diagnostic work by eliminating the long uncertain wait for slow lactose fermentation to occur.

SUMMARY

1. A new multiple-carbohydrate medium was devised as a means of differentiating paracolon bacilli as a group from Salmonella organisms. The medium contained 0.5 per cent each of adonitol, aesculin, salicin, and sucrose. From the initial letters of the four component carbohydrates the name AASS medium was invented.

2. The AASS medium was a rapid means of differentiating paracolon from Salmonella cultures on the basis of fermentation of one or more of four carbohydrates which are utilized by paracolon bacilli but not by Salmonella.

3. Ability to produce indole was demonstrated accurately in the AASS medium and was an additional differential characteristic of great value.

4. Eighty per cent of the paracolon strains were detected within twenty-four hours by the use of this medium.

REFERENCES

1. Monteverde, J. J.: Relaciónes Serologicas Entra las Bacterias del Genero Salmonella y las Bacterias Fermentadoras Lentas de Lactosa, Rev. Fac. Agron. Vet. B. Aires **10**: 370, 1943.
2. Russell, F. F.: The Isolation of Typhoid Bacilli From Urine and Feces With the Description of a New Triple Sugar Tube Medium, J. Med. Research **25**: 217, 1912.
3. Hajna, A. A.: Triple-Sugar Iron Agar Medium for the Identification of the Intestinal Group of Bacteria, J. Bact. **49**: 516, 1945.

4. Sulkin, S. E., and Willett, J. C.: A Triple Sugar-Ferrous Sulfate Medium for Use in Identification of Enteric Organisms, J. Lab. & Clin. Med. **25**: 649, 1940.
5. Felsenfeld, O., and Young, V. M.: A New Medium for the Differentiation of Salmonella and Paracolon Organisms, Am. J. Clin. Path., Tech. Sect. **8**: 26, 1944.
6. Krumwiede, C., and Kohn, L.: A Triple Sugar Modification of the Russell Double Sugar Medium, J. Med. Research **37**: 225, 1918.
7. Kligler, I. J.: A Simple Medium for the Differentiation of the Members of the Typhoid-Paratyphoid Group, Am. J. Pub. Health **7**: 1042, 1917.
8. Friewer, F., and Shaughnessy, H. J.: Lead Semi-Solid Agar; A Medium for Use in the Identification of the Enteric Group of Bacteria, Am. J. Clin. Path., Tech. Sect. **8**: 1, 1944.
9. Weil, A. J., Black, J., and Farsetta, K.: The Serological Types of Shigella Paradysenteriae (Flexner); Types With Single Primary Antigen, J. Immunol. **49**: 321, 1944.
10. Rustigian, R., and Stuart, C. A.: Decomposition of Urea by Proteus, Proc. Soc. Exper. Biol. & Med. **47**: 108, 1941.

BOOK REVIEWS

Journal of the History of Medicine and Allied Sciences. Edited by *George Rosen*. Published quarterly by Henry Schuman, New York, N. Y. Subscription rate $7.50 in the United States, Canada, and Latin America; $8.50 elsewhere. Single copies $2.50.

Ambulatory Proctology. By *Alfred J. Cantor*, M.D., Associate Proctologist, Kew Gardens General Hospital, Long Island, New York; formerly Assistant Attending Gastroenterologist, Queens General Hospital, New York; and Assistant Adjunct Proctologist, Hospital for Joint Diseases, New York. With a foreword by Beaumont S. Cornell, M.D., Editor, American Journal of Digestive Diseases. Paul B. Hoeber, Inc., New York, 1946. Price $8.00. Cloth with 524 pages and 110 illustrations.

Modern Management in Clinical Medicine. By *Frederick K. Albrecht*, M.D., S. A. Surgeon, United States Public Health Service; Kansas State Tuberculosis Consultant; formerly Clinical Director, United States Marine Hospital, Baltimore, Md. Williams & Wilkins Company, Baltimore, 1946. Price $10.00. Cloth with 1238 pages.

Physical Methods of Organic Chemistry. Vol. 11. Edited by *Arnold Weissberger*. Interscience Publishers, Inc., New York, 1946. Price $8.50. Cloth with 630 pages.

Principles and Practice of Tropical Medicine. By *L. Everard Napier*, Companion of the Order of the Indian Empire; Fellow of the Royal College of Physicians of London; formerly Director and Professor of Tropical Medicine, Calcutta School of Tropical Medicine; Consultant to the Secretary of War; Visiting Lecturer on Tropical Medicine, Army Medical School; Visiting Lecturer on Tropical Medicine, Harvard Medical School, Boston; formerly Visiting Professor of Tropical Medicine, Tulane University, New Orleans; formerly Visiting Professor of Medicine, New York University. The Macmillan Company, New York, 1946. Price $11.00. Cloth with 918 pages.